US009149516B2

(12) **United States Patent**
Livingston et al.

(10) **Patent No.: US 9,149,516 B2**
(45) **Date of Patent: *Oct. 6, 2015**

(54) **POLYVALENT CONJUGATE VACCINE FOR CANCER**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 109 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **13/951,795**

(22) Filed: **Jul. 26, 2013**

(65) **Prior Publication Data**

US 2014/0037673 A1 Feb. 6, 2014

Related U.S. Application Data

(63) Continuation of application No. 13/314,521, filed on Dec. 8, 2011, now Pat. No. 8,540,964, which is a continuation of application No. 12/262,729, filed on Oct. 31, 2008, now Pat. No. 8,092,780, which is a continuation of application No. 10/752,945, filed on Jan. 6, 2004, now Pat. No. 7,479,266, which is a continuation-in-part of application No. PCT/US02/21348, filed on Jul. 5, 2002.

(60) Provisional application No. 60/303,494, filed on Jul. 6, 2001, provisional application No. 60/347,231, filed on Jan. 10, 2002.

(51) **Int. Cl.**
A61K 39/385 (2006.01)
A61K 39/00 (2006.01)

(52) **U.S. Cl.**
CPC ... ***A61K 39/0011*** (2013.01); *A61K 2039/55577* (2013.01); *A61K 2039/6081* (2013.01); *A61K 2039/627* (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

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(57) **ABSTRACT**

This invention provides a polyvalent vaccine comprising at least two conjugated antigens selected from a group containing glycolipid antigen, polysaccharide antigen, mucin antigen, glycosylated mucin antigen and an appropriate adjuvant. This invention also provides a multivalent vaccine comprising at least two of the following: glycosylated MUC-1-32mer, Globo H, GM2, Le^y, Tn(c), sTN(c), and TF(c). This invention provides the vaccine above, wherein the adjuvant is saponin-based adjuvant. This invention provides a method for inducing immune response in a subject comprising administering an effective amount of the vaccine above to the subject. Finally, this invention provides a method for treating cancer in a subject comprising administering an appropriate amount of the vaccine above to the subject.

8 Claims, No Drawings

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POLYVALENT CONJUGATE VACCINE FOR CANCER

The application disclosed herein is a continuation-in-part of International Application No. PCT/US02/21348, filed Jul. 5, 2002, which claims priority of U.S. Ser. 60/303,494, filed on Jul. 6, 2001 and U.S. Ser. No. 60/347,231, filed on Jan. 10, 2002, the contents of which are hereby incorporated by reference into this application.

Throughout this application, various references are referred to. Disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains.

The invention disclosed herein was made with United States government support under NIH Grant Nos. CA33049 and CA52477 from the United States Department of Health and Human Services. Accordingly, the United States Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

Tumor-specific antigens have been identified and pursued as targets for vaccines. Previous work from the inventors' has shown that monovalent vaccines utilizing the tumor antigens Globo H, Lewis^y, GM2, glycosylated MUC-1, Tn(c), sTn(c), or TF(c) conjugated to KLH to be safe with local erythema and edema but minimal systemic toxicities. As a result of vaccination with these monovalent vaccines, most patients generated specific high titer IgM or IgG antibodies against the respective antigen-KLH conjugates. The present invention provides a polyvalent vaccine wherein the components of the monovalent vaccines are combined and administered with an adjuvant as treatment for cancer.

SUMMARY OF THE INVENTION

The invention disclosed herein provides a polyvalent vaccine comprising at least two conjugated antigens selected from a group containing glycolipid antigen, polysaccharide antigen, mucin antigen, glycosylated mucin antigen and an appropriate adjuvant. This invention also provides the multivalent vaccine, comprising glycosylated MUC-1-32mer, Globo H, GM2, Le^y, Tn(c), and TF(c). This vaccine may comprise glycosylated MUC-1-G5, Globo H, GM2, Le^y, Tn(c), sTN(c), and TF(c). This invention provides the vaccine above, wherein the adjuvant is saponin-based adjuvant.

This invention also provides a method for inducing immune response in a subject comprising administering an effective amount of the vaccine above to the subject. Finally, this invention provides a method for treating cancer in a subject comprising administering an appropriate amount of the vaccine above to the subject.

DETAILED DESCRIPTION OF THE INVENTION

The invention disclosed herein provides a polyvalent vaccine comprising at least two conjugated antigens selected from a group containing glycolipid antigen, polysaccharide antigen, mucin antigen, glycosylated mucin antigen and an appropriate adjuvant.

The glycolipid includes but is not limited to Globo H, a Lewis antigen and a ganglioside. The Lewis antigen includes but is not limited to Le^y and sialyl Le^a. The ganglioside includes fucosylated GM1, GM2, GD2, or GD3. In another embodiment, the mucin is a MUC peptide. In a further embodiment, the MUC peptide is MUC-1, MUC-2 or MUC-16. The polysaccharide antigen includes but is not limited to Tn(c), sTn(c), TF(c), and polysialic acid.

This invention provides a bivalent, trivalent, tetravalent, pentavalent, hexavalent, and heptavalent vaccine. The vaccine comprises at least two conjugated antigens selected from a group containing glycolipid antigen, polysaccharide antigen, mucin antigen, glycosylated mucin antigen and an appropriate adjuvant.

In an embodiment, the hexavalent vaccine comprises glycosylated MUC-1-32mer, Globo H, GM2, Le^y, Tn(c), and TF(c). In a further embodiment, the range of MUC-1-32mer is from about 0.1 to 30 ug. In yet another embodiment, the range of Globo H is from about 0.1 to 100 ug. In still a further embodiment, the range of GM2 is from about 0.1 to 100 ug. In an additional embodiment, the range of Le^y is from about 0.1 to 60 ug. In a further embodiment, the range of Tn(c) is from about 0.1 to 100 ug. In an additional embodiment, the range of TF(c) is from about 0.1 to 30 ug.

In a separate embodiment, the adjuvant is saponin based. The adjuvant includes QS21 and GPI-0100. In an embodiment, the range of QS21 is from about 25 to about 200 ug. In another embodiment, QS21 is about 100 ug. In a separate embodiment, the adjuvant is GPI-0100 with a range from about 1 to 25 mg. In an embodiment, GPI-0100 is about 10 mg.

This invention provides a heptavalent vaccine comprising at least two conjugated antigens selected from a group containing glycolipid antigen, polysaccharide antigen, mucin antigen, and glycosylated mucin antigen and an appropriate adjuvant. In an embodiment, the vaccine comprises glycosylated MUC-1-G5, Globo H, GM2, Le^y, Tn(c), sTN(c), and TF(c). In another embodiment, the range of MUC-1-G5 is from about 0.1 to 30 ug. In a further embodiment, the range of Globo H is from about 0.1 to 100 ug. In another embodiment, the range of GM2 is from about 0.1 to 100 ug. In still another embodiment, the range of Le^y is from about 0.1 to 60 ug. In an embodiment, the range of Tn(c) is from about 0.1 to 100 ug. In a further embodiment, the range of sTn(c) is from about 0.1 to 100 ug. In yet another embodiment, the range of TF(c) is from about 0.1 to 30 ug.

This invention provides the vaccine above, wherein the adjuvant is saponin-based adjuvant. These saponin-based adjuvants include but are not limited to QS21 and GPI-0100.

In an embodiment, the range of QS21 is from about 25 to 200 ug. In another embodiment, the QS21 is about 100 ug. In a separate embodiment, the adjuvant is GPI-0100 and the range is from about 1 to 25 mg. In a preferred embodiment, GPI-0100 is about 10 mg.

This invention provides a polyvalent vaccine comprising a conjugated glycosylated antigen, a conjugated ganglioside antigen and an appropriate adjuvant, wherein the antigens are conjugated to a carrier. In an embodiment, the carrier is Keyhole Limpet Hemocyanin (KLH).

This invention provides the polyvalent vaccine above comprising at least two conjugated antigens selected from a group containing glycolipid antigen, polysaccharide antigen, mucin antigen, and glycosylated mucin antigen and an appropriate adjuvant for cancer. In an embodiment, the cancer is prostate, breast or ovarian cancer.

This invention also provides a method for inducing immune response in a subject comprising administering an effective amount of the above vaccine to the subject.

Furthermore, this invention provides a method for treating cancer in a subject comprising administering an appropriate amount of the above vaccine to the subject.

This invention also provides a composition comprising the above vaccine and a carrier.

This invention also provides a pharmaceutical composition comprising the above vaccine and a pharmaceutically acceptable carrier.

In addition, the invention provides a vaccine for small cell lung cancer comprising at least two conjugated antigens selected from the group containing Globo H, fucosylated GM1, GM2, GD2, GD3, sialyl Le^a and polysialic acid. This invention also provides a method for inducing immune response in a subject bearing small cell lung cancer comprising administering an effective amount of the above vaccine to the subject. This invention furthermore provides a method for treating a subject bearing small cell lung cancer comprising administering an effective amount of the above vaccine to the subject.

In addition, this invention provides the above vaccine, further comprising an antigen selected from a group containing CA125, or a portion thereof, KSA peptide or protein, and PSMA, or a portion thereof.

This invention includes the above vaccines which further comprise other antigens which can induce antibody and/or immune response. As illustrated throughout the specification, the antigen used may be modified to increase its immunogenicity. Said antigens include but are not limited to CA125, or a portion thereof, KSA peptide or protein, and PSMA, or a portion thereof. As can be easily appreciated by the ordinary skilled artisan, only a portion of the antigen may be required for induction of immune response from a subject.

As stated herein, subjects are organisms which have immune response. The subject includes but is not limited to humans. Said subject could be domestic animals, such as dogs and cats.

This invention further provides the above compositions and a pharmaceutically acceptable carrier, thereby forming pharmaceutical compositions.

This invention also provides a pharmaceutical composition comprising a combination as described above and a pharmaceutically acceptable carrier. For the purposes of this invention, "pharmaceutically acceptable carriers" means any of the standard pharmaceutical carriers. Examples of suitable carriers are well known in the art and may include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution and various wetting agents. Other carriers may include additives used in tablets, granules and capsules, etc. Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gum, glycols or other known excipients. Such carriers may also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well-known conventional methods.

The invention will be better understood by reference to the Experimental Details which follow, but those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative, and are not meant to limit the invention as described herein, which is defined by the claims which follow thereafter.

EXPERIMENTAL DETAILS

First Series of Experiments

Polyvalent (Hexavalent) Conjugate Vaccine for Prostate, Breast, Ovarian and Small Cell Lung Cancer

Tumor-specific antigens have been identified and pursued as targets for vaccines. The inventors' previous work has shown that monovalent vaccines utilizing the tumor antigens Globo H, $Lewis^y$, GM2, glycosylated MUC-1, Tn(c), or TF(c) conjugated to KLH to be safe with local erythema and edema but minimal systemic toxicities. As a result of vaccination with these monovalent vaccines, most patients generated specific high titer IgM or IgG antibodies against the respective antigen-KLH conjugates. The present invention provides a hexavalent vaccine wherein the components of the monovalent vaccines are combined and administered with an adjuvant as treatment for prostate, breast, ovarian and small cell lung cancer.

A vaccine consisting of a unique combination of six tumor antigens administered with a saponin immunological adjuvant QS-21 or GPI-0100. The antigens are glycosylated MUC-1-32mer, Globo H, GM2, Le^y, Tn(c), and TF(c). In each case the antigen is conjugated to Keyhole Limpet Hemocyanin (KLH).

The preferred ranges of the antigen and adjuvant doses are as follows:

Glycosylated MUC-1-32mer: 0.1 to 30 μg;
Globo H, 0.1 to 100 μg;
GM2: 0.1 to 100 μg;
Le^y: 0.1 to 60 μg;
Tn(c): 0.1 to 10 μg;
TF(c): 0.1 to 30 μg;
QS-21: 100 μg;
GPI-0100: 1 or 25 mg.

Example 1

A Phase I Multivalent Conjugate Vaccine Trial for Patients with Biochemically Relapsed Prostate Cancer

1.0 PROTOCOL SUMMARY
2.0 OBJECTIVE
3.0 BACKGROUND AND RATIONALE
4.0 VACCINE PREPARATION
5.0 IMMUNIZATION SCHEDULE
6.0 PRE- AND POST-THERAPY EVALUATION
7.0 RESPONSE CRITERIA
8.0 BIOSTATISTICAL CONSIDERATIONS
9.0 REFERENCES

1.0 Protocol Summary:

This is a phase I pilot trial designed to assess safety using a multivalent conjugate vaccine. This trial is based on the results of eight dose-seeking phase I monovalent glycoprotein and carbohydrate conjugate vaccine trials which have been shown to be consistently immunogenic in man. These trails also allowed us to screen candidate antigens for their ability to generate high titer specific antibodies against the immunizing antigen. This vaccine will consist of the highest dose of synthetic glycoprotein and carbohydrate antigens shown to elicit high titer IgM and IgG antibodies in patients with biochemically relapsed prostate cancer. The inventors' previous work has shown the monovalent vaccines to be safe with local erythema and edema but minimal systemic toxicities. Among the antigens to be included in the multivalent vaccine are carbohydrate antigens Globo H and GM2 and the glycoprotein antigens glycosylated MUC-1-32mer, Lewisy, Tn(c), and TF(c). The patient populations to be targeted are those patients who have failed primary therapies such as prostatectomy or radiation or have been on intermittent hormonal therapy and have remained hormonally sensitive in the absence of radiographic disease. These populations must have as the sole indication of disease progression, a rising PSA. The inventors' data from approximately 160 men who

participated in earlier monovalent vaccine trials against the aforementioned antigens have shown that a treatment effect in the form of a decline in PSA log slopes compared with pretreatment values could be seen in patients with minimal tumor burden. A phase III double blind randomized trial with two hundred forty patients is planned based on the safety data accrued form this proposed phase I trial. The primary endpoint of the study will be the ability to assess the safety of the vaccine and the humoral response to a multivalent conjugate. Secondary endpoints will be to evaluate post-therapy changes in PSA.

2.0 Objectives:

2.1 The primary endpoints of the study are:

2.1.1 To determine the safety of a multivalent conjugate vaccine in patients with prostate cancer who have biochemically relapsed following primary therapies such as surgery or radiation.

2.1.2 Measure the antibody response against the individual components of the vaccine and to correlate the response to subsequent clinical course.

2.2. The secondary endpoints will be:

2.2.1 To assess post-immunization changes in prostate specific antigen levels and other objective parameters of disease (radionuclide bone scan and/or measurable disease if present.

3.0 Background and Rationale:

3.1 Prostate Cancer:

Over 180,000 cases of prostate cancer will be diagnosed in the United States in 2000.[1] Of these, 30-35% will present with tumors beyond the confines of the gland, while an additional 25% will develop metastases in the course of the disease despite local therapies. In these cases, a rising PSA antedates the development of overt metastases by a median of 12-24 months. Androgen ablation is the standard treatment with upwards of 70% of cases showing a normalization of an abnormal PSA after therapy. When to initiate treatment remains an area of controversy and there is no evidence that deferring therapy compromises outcomes. This observation, coupled with the fact that most patients relapsed within a median of 12-18 months[2], and that most men can not tolerate the side effects of castration including impotency, weight gain and hot flashes, has led to the search for alternative therapies. One such approach involves enhancing the body's own immune system as a means to treat local disease and prevent disease progression. PSA monitoring allows the identification of patients with low-volume disease, in whom an immunostimulatory approach may be more efficacious relative to a heavily pretreated, symptomatic population with large tumor burdens. Vaccinations represent a safe intervention with minimal toxicities that can be given as an adjuvant to surgery or radiation therapy in men at risk for systemic relapse. They can also be offered to men with minimal tumor burdens who are progressing and who are not willing to accept toxicities of hormonal therapy or chemotherapy. Because hormonal status may effect antigen expression and regulation, we propose to enroll patients with different hormone sensitivities. This will include patients who have not received hormonal therapy or have been on intermittent hormonal therapy.

3.2 PSA as an Endpoint for Clinical Trials:

The availability of serum PSA determinations provides a unique trial design for testing new therapies rapidly as changes in PSA levels over time correlate well with clinical outcomes.[3] This relationship holds for both hormone-naïve and hormonally relapsed disease. Once sequential elevations in PSA are documented in the setting of castrate testosterone levels, clinical symptoms develop in a median of 3-6 months. This observation justifies treatment in the setting of rising PSA values, using post-therapy changes in PSA as the outcome measure. With this design, therapeutic approaches that do not produce a defined degree of decline in PSA on multiple determinations for a defined duration (vide infra) are not evaluated further.[2]

3.3 Immunologic Approaches:

Augmentation of the immune response to cancer can be attempted by two basic approaches: non-specific immunopotentiation which constitutes the bulk of past and current efforts at cancer immunotherapy, and specific immunization which has not really been evaluated in the treatment of cancer but has contributed much to the control of infectious diseases. It is the knowledge of microbial antigens which has permitted the development of successful specific immunization against infections. The lack of availability of well-defined human cancer antigens, on the other hand, has prevented exploration of specific immunization in the context of cancer as it should be explored, using vaccines of defined cancer-restricted antigenicity and demonstrating their immunogenicity in cancer patients.

3.3.1 The Role of Carbohydrates and Mucins in Prostate Cancer:

Carbohydrate antigens have proven to be clinically relevant and (aside from vaccines against toxins) are the only defined bacterial antigens used in vaccines against bacterial pathogens. Immunization with carbohydrate antigens has also resulted in directed antibody responses against human tumor cells (reviewed in [4]), presumably because these antibodies are known to mediate antibody-dependent cell-mediated and complement mediated lysis of tumor cells, complement-induced inflammation, and phagocytosis by the reticulo-endothelial system. The inventors' previous study in prostate cancer focused on defining the antigens expressed on the surface of prostate cancer cells.

3.3.2 Results with Eight Monovalent Phase I Trials Using Glycoprotein Peptides and Carbohydrate Antigens.

Immunohistochemistry using well-defined monoclonal antibodies against glycoprotein and carbohydrate antigens have shown that primary and metastatic prostate carcinoma specimens express these heretofore unknown antigens and that these molecules can serve as targets for immune recognition. We have studied two mucin peptide antigens, MUC-1 and MUC-2 conjugated to KLH and given with the immune adjuvant, QS21 in the phase I setting as a dose escalating trial with 10, 100 and 3 μg. Patients received five subcutaneous vaccines over the course of twenty-six weeks at weeks 1, 2, 3, 7, and 19. Twenty patients were treated in the MUC-1-KLH-QS21 trial and fifteen were treated with MUC-2-KLH-QS21 trial. All patients developed high titer IgM and IgG antibodies specific for the immunizing peptide. Antibody titers rose by week 7 and declined usually by week 19, the time of the fifth and final vaccine. Unexpectedly, a treatment effect was observed after the vaccine trial was completed in the form of a declining PSA log slope compared with pretreatment values in approximately two-thirds of patients. In many patients, the slope began to show a decline by week 38 with subsequent declines by week 60. The initial decline corresponded to the rise of antibodies following the last immunization received at week 19. Five patients who were treated with the MUC-1-KLH conjugate in 1996 continue to have stable PSA log slopes without radiographic evidence of disease. Patients who were treated with MUC-2-KLH conjugate also demonstrated a similar treatment effect, however, the trial has not as yet reached maturity. The vaccines were found to be safe with erythema, tenderness and edema at the injection site. No evidence of autoimmunity or systemic toxicity was observed.

3.3.3 Experience with Glycolipid and Carbohydrate Antigens.

Eighteen patients have undergone immunization with Globo H, a glycolipid antigen expressed on prostate cancer cells. This is the first purely synthetic complex carbohydrate antigen used for immunization in man capable of generating high titer specific antibodies (median peak titer 1:320, IgG median titer 160) capable of mediating complement lysis of tumor cells. Several patients generated IgM antibody titers of 1:20,480). Patients were immunized with 10, 30 100 or 3 μg of Globo H-KLH plus QS21 over twenty-six weeks. Of the patients immunized with this vaccine, six remain active with stable PSA log slopes and no radiologic evidence of evidence of disease over the last 2½ years. This vaccine was found to be safe with no evidence of systemic toxicity. Ganglioside antigens (acidic glycosphingolipids expressing sialic acid at one end and ceramide at the other) was also investigated in a trial comparing the immunogenicity of higher doses of QS21. Using GM2-KLH at 30 μg, a dose previously established in melanoma trials, 18 patients were immunized with either the GM2 conjugate plus QS21 at the standard dose of 100 μg or QS21 at 225 μg. Because of its potential for systemic toxicity, the latter vaccine was given as three separate immunizations to three separate sites as GM2-KLH at 10 μg plus QS21 at 75 μg subcutaneously. No difference in antibody titers were observed in two groups of patients; although two patients from the group given the higher dose of QS21 experienced grade II myalgias. Several patients also exhibited a decline in PSA log slopes but there did not appear to be any difference between groups with regard to treatment effects.

4.0 Vaccine Preparation

Globo H, Lewis^y, Tn(c), TF(c) are synthesized in the laboratory of Bio-Organic Chemistry headed by Dr. Samuel Danishefsky. MUC-1-32mer is synthesized in the Core Peptide Synthesis Facility of The Rockefeller Research Laboratories under the aegis of Dr. Paul Tempst. It is glycosylated with Tn by Dr. Henrik Clausen at the University of Copenhagen, Copenhagen, Denmark. GM2 is extracted from rabbit brains by Progenics, Inc., Tarrytown, N.Y.

4.1 Globo H, MUC-1-32mer, GM2, Lewis^y, Tn(c) and TF(c)-KLH conjugation:

The above antigens will be covalently attached to KLH in Dr. Livingston's laboratory. Antigen-KLH ratios between 150/1 and 800/1 assuming a KLH molecular weight of 5×10^6 will be accepted. Gels will be performed and western blot analysis will be conducted with each lot of antigen-KLH for comparison to future lots. Sterility and safety testing with each lot plus QS21, at >50 times the dose/meter^2 to be used in clinical trials will be performed. No growth in culture and no adverse reaction in mice or Guinea pigs (including weight loss of 10% or more) will be tolerated. Two or more mice will be immunized with each antigen-KLH batch on 2-3 occasions at 1-2 week intervals and post immunization sera tested. Antibody titers of 1/200 or greater against antigen and 1/40 by IA or FACS staining of >25% of antigen positive cells will be accepted as proof that the construct has the appropriate immunogenicity.

4.2 Antigen Doses:

Based on previous vaccine trials in prostate cancer patients, the following doses have been established for the multivalent trial: glycosylated MUC-1-32mer, 3 μg; Globo H, 10 μg; GM2, 10 μg; Le^y, 10 μg; Tn(c), 3 μg; and TF(c), 3 QS21 will be used at 100 μg as no significant difference in immunogenicity was observed with doses as high as 225 μg.

4.3 Safety Testing:

Samples from the materials are sent for sterility and safety testing. Immunogenicity of the individual peptides/carbohydrates have been previously confirmed in mice.

5.0 Immunization Schedule:

5.1 Patient Selection:

All patients with evidence of biochemical relapse will be considered. Hormonal status will be recorded on the basis of serum testosterone levels as follows: Patients who have progressed after primary surgery or radiation (with or without neo-adjuvant androgen ablation) who have non-castrate levels of testosterone (>50 ng/ml) will be eligible.

5.2 Interval:

The immunization schedule that we will utilize was derived from the inventors' studies with other glycoprotein and carbohydrate conjugate vaccines in patients with melanoma, colon and breast cancers.

5.3 Treatment Schedule and Dose:

Fifteen patients will be treated with specified doses of each carbohydrate or peptide constituent as has been determined previously based on earlier monovalent trials completed. QS21 will be administered at the standard dose of 100 ug. Sites: The vaccine conjugate will be administered subcutaneously to random sites on the upper arms and upper legs.

5.4 Dose Modifications:

If a patient experiences a Grade III or greater local or Grade II or greater systemic toxicity at any time a decrease by 50% in all components of future vaccinations will be administered for that patient.

6.0 Pre- and Post-Therapy Evaluation:

6.1 Outcomes:

The study evaluation will include parameters to assess the safety of the vaccine, antitumor effect, as well as assessments of immune function. Interval safety assessments will include the Patient Diary. An overall antitumor assessment will be performed during weeks 13 and 26. If the patient has not demonstrated progression of disease at week 13 or 26, he will continue on protocol. Upon completion of the trial, he will be monitored every 3 months with bloodwork and imaging studies for the next 2 years or until disease progression.

6.2 Safety and Antitumor Effects:

	STUDY WEEK								
Clinical:	O[a,c]	1	2	3	7	9	13	19	26
Performance Status	X[b]	—	—	X	X	X	X	X	X[h]
Interval Hx & PE	X[b]	—	—	X	X	X	X	—	X
CBC, Diff, Plt.	X	—	—	X	X	—	X	—	X
CMP[d], LDH	X	—	—	X	—	—	X	—	X
Uric acid, Phosphorus	X	—	—	X	—	—	X	—	X
Prothrombin time	X	—	—	—	—	—	—	—	—
PSA, Ac. Phos	X	—	—	X	X	—	X	—	X
Testosterone	X	—	—	—	—	—	—	—	—
U/A	X	—	—	X	X	X	X	—	X
Stool guaiac	X	—	—	—	X	—	—	—	X
Pathology Review[e]	X	—	—	—	—	—	—	—	—
Imaging:[f]									
Chest X-ray	X	—	—	—	—	—	X	—	X
Bone scan	X	—	—	—	—	—	X	—	X
CT Scan or MRI	X	—	—	—	—	—	X	—	X
Overall response assessments[g]	—	—	—	—	—	—	X	—	X

-continued

	STUDY WEEK								
Clinical:	O[a,c]	1	2	3	7	9	13	19	26
Consent for Pathologic Correlates[i]	X								

[a]Baseline studies prior to immunization.

[b]Within 7 days of the first immunization.

[c]Within 15 days of starting treatment for biochemical studies; 30 days for imaging studies. Includes total bilirubin, SGOT, LDH, Alkaline Phosphatase, Creatinine, BUN.

[d]CMP Includes total bilirubin, SGOT, ALT, Sodium, Potassium, Chloride, CO_2, Calcium, Glucose, Total Protein, Abumin, Alkaline Phosphatase, Creatinine, BUN.

[e]Patients will be asked to obtain tissue blocks from previous diagnostic/therapeutic procedures will be obtained and the patient's tumor evaluated for the presence of the antigens by immunohistochemistry. The presence of any antigen on paraffin material is not a criterion for entry and no biopsy procedures will be performed specifically for enrollment.

[f]Abdominal and pelvic CT scans with and without contrast, chest x-ray and any other tests deemed necessary to document evaluable disease.

[g]Overall response assessment includes the repetition of abnormal imaging and biochemical studies used to assess disease, and in selected cases, immune function.

[h]Repeat at 3 month intervals for 2 years or until disease progression is documented.

[i]Patients will be asked to sign a separate consent for pathologic correlative studies under IRB [90-40: Dr. H. Scher, P. I. - Molecular correlations in human prostate cancer].

6.3 Immune Function:

	STUDY WEEK								
	0	1	2	3	7	9	13	19	26
VACCINATION*	—	1	2	3	4	—	—	5	—
B-CELL TESTING	—	1	2	3	4	5	6	7	8

*No skin tests will be performed as previous trials indicated that there is minimal or no reactivity with intradermal administration of the antigens studied.

Antibody Response:

Peripheral blood (30 cc) will be drawn prior to vaccine immediately before each vaccination, as well as weeks 9, 13, and 26 to assess B-cell function. Thereafter, blood will be drawn at 3-month intervals (up to one year from the first vaccination), or as long as detectable immunity against the antigens persist. Depending on the antibody response, additional testing involving proliferation and cloning may be performed at a later date. The patients' sera will be tested by ELISA for antibodies against purified antigens as well as a variety of cell lines expressing (or not) the antigens included in the vaccine.

7.0 Response Criteria:

7.1 Patients WITHOUT bi-dimensionally measurable disease are evaluable by post-therapy changes in PSA as follows:

7.1.1 Complete Response (CR): Normalization of the PSA (≤1.0 or 2.0 as defined in 4.1.1) for 3 successive evaluations at least 2 weeks apart.

7.1.2 Partial Response (PR): Decrease in PSA value by ≥50% above baseline (without normalization) for 3 successive evaluations.

7.1.3 Stabilization (STAB): Patients who do not meet the criteria for PR or PROG for at least 90 days will be considered stable.

7.1.4 Progression (PROG): Three consecutive increases in PSA, to >50% above baseline.

7.2 Duration of response: Non-measurable disease: Time from initiation of therapy until a 50% increase from the PSA nadir value is documented on three successive determinations.

8.0 Biostatistical Consideration

8.1 This is an exploratory study to study the safety of a multivalent conjugate vaccine which will be taken to phase III clinical trials. Patients with prostate cancer who have experienced a PSA recurrence after radical prostatectomy or radiation therapy are eligible. All fifteen patients will receive the same dose. The dose is based on previous vaccine trials in prostate cancer patients, the following doses have been established for the multivalent trial: glycosylated MUC-1-32mer, 3 μg; Globo H, 10 μg; GM2, 10 μg; Le^y, 10 μg; Tn(c), 3 μg; and TF(c), 3 μg. QS21 will be used at 100 μg as no significant difference in immunogenicity was observed with doses as high as 225 μg. Subjects will be followed for two years or until the development of metastatic disease. Bone scan and CT scans (or MRI where clinically appropriate) will be performed approximately at week 13, 26, and approximately every 3 months thereafter until the development of metastatic disease. In addition, PSA measurements will be obtained at weeks 3, 7, 13, 26, and every approximately 3 months thereafter in order to study the effect of the vaccine on the probability of developing metastatic disease and the effect of the vaccine on PSA slope over time, respectively.[24]

8.2 In order to be eligible for the study, patients must have a rising PSA following radical prostatectomy or radiation therapy. This detection of PSA following treatment must occur within two years. Using the ASTRO definition, three consecutive PSA rises are considered a biochemical failure after radical prostatectomy or radiation therapy. The date of failure should be the midpoint between the postsurgical (or postirradiation) nadir PSA and the first of the three consecutive rises.[25] In addition, patients must have a PSA doubling time (DT) less than 5 months. PSA doubling time is determined prior to treatment and is equal to ln(2) divided by the least squares derived slope of log PSA over time (log PSA slope >0.15).[26] The time interval in which PSA DT will be based will consist of a minimum of three PSA measurements in a twelve-month interval prior to randomization. Patients who meet this requirement are considered at a higher risk for metastatic disease and will be eligible for this trial.

8.3 The primary objective of this study is to determine the safety and the humoral response of the multivalent vaccine in preparation for the phase III trial. The primary endpoint will be the time to radiographic progression of disease. The secondary objective is to study the rate of change in PSA over time.

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Example 2

Hexavalent Vaccine Immunogenicity Trial 1N Mice

Methods

Serological Analyses

1. ELISA (Enzyme-Linked Immunosorbent Assay):

ELISA assays were performed as described below. Antigen in ethanol or in 0.1 M carbonate buffer (pH 11) were coated on ELISA plates at 0.2 μg/well for glycolipids and 0.1 μg/well for peptides. Serially diluted antiserum was added to each well and alkaline phosphatase-conjugated goat anti-mouse IgM or anti-mouse IgG was added at a dilution of 1:200 (Southern Biotechnology Associates, Inc, Birmingham, Ala.). Goat anti-mouse IgG and IgM conjugated with alkaline phosphatase obtained from Kierkegaard and Perry Labs, (Gaithersburg, Md.) were used as second antibodies. ELISA titer is defined as the highest dilution yielding an absorbance of 0.1 or greater over that of normal control mouse sera.

2. Cell Surface Reactivity Determined by FACS:

The cell surface reactivity of immune sera was tested on human cell lines. Single cell suspensions of 2×10^5 cells/tube were washed in PBS with 3% fetal calf serum (FCS) and 0.01M NaN_3 and incubated with 20 μl of 1:20 diluted sera or monoclonal antibody mAb for 30 min on ice. After two washes with 3% FCS in PBS, 20 μl of 1:15 diluted goat anti-mouse IgM or IgG-labeled with fluorescein-isothiocyanate (FITC, Southern Biotechnology Associates Inc. Birmingham, Ala.) was added, and the mixture incubated for 30 min. After a final wash, the positive population and mean fluorescence intensity of stained cells were differentiated using FACScan, Becton & Dickinson Immunocytometry, San Jose, Calif.

Appendix B

Hexavalent Vaccine Immunogenicity Trial in Mice
Sep. 20, 2000

Four female CB6F1 mice were vaccinated weekly for three weeks with Hexavalent vaccine

(Hexavalent vaccine in Polyval-KLH conjugate plus 20 ug QS21 per mouse).

The injections were SC, at 2 sites, with 95 ul/site.

[Vial labeled Polyval in Polyval-KLH conjugate plus 100 ug QS21/1.0 ml. Total vol. 1.0 ml. Lot #081100]

Pre-vaccination sera was drawn from each mouse.

Sera was drawn again at 10 days post third vaccination.

The mice were weighed prior to and post vaccination (at 24 hr post, at 48 hr post, at one week post and at 2 weeks post).

For controls, the following monoclonal antibodies were used:

VK9	anti-Globo-H
BR96	anti-Le^y
HMFG1	anti-MUC1
αTn Ab	anti-Tn
αGM2 Ab	anti-GM2
49H.8	anti-TF

Serology

ELISA plates were coated with 0.1 ug/well of one of the following antigens: GloboH-ceramide, GM2 (IgM), MUC1G5, Tn-'HSA, Tf-'HSA

ELISA plates were coated with 0.2 ug/well of one of the following antigens: GM2 (IgG), Ley

Sera was tested at an initial dilution of 1:40, with subsequent 2-fold dilutions (with the exception of GloboH for which 3-fold dilutions were used).

FACS analysis was performed on two cell lines: MCF7 and LSC (5×105 cells per tube).

Sera was added at a 1:20 dilution (25 ul/tube).

Each post 3rd vacc. sera was set against its corresponding pre-sera (each pre-sera was set at 10%).

Second Series of Experiments

Polyvalent (Heptavalent) Conjugate Vaccine for Prostate, Breast, Ovarian and Small Cell Lung Cancer

Tumor-specific antigens have been identified and pursued as targets for vaccines. The inventors' previous work has shown that monovalent vaccines utilizing the tumor antigens Globo H, Lewis^y, GM2, glycosylated MUC-1, Tn(c), sTn(c), or TF(c) conjugated to KLH to be safe with local erythema and edema but minimal systemic toxicities. As a result of vaccination with these monovalent vaccines, most patients generated specific high titer IgM or IgG antibodies against the respective antigen-KLH conjugates. The present invention provides a heptavalent vaccine wherein the components of the monovalent vaccines are combined and administered with an adjuvant as treatment for prostate, breast, ovarian and small cell lung cancer.

A vaccine consisting of a unique combination of seven tumor antigens administered with a saponin immunological adjuvant QS-21 or GPI-0100. The antigens are glycosylated MUC-1-G5, Globo H, GM2, Le^y, Tn(c), sTn(c), and TF(c). In each case the antigen is conjugated to Keyhole Limpet Hemocyanin (KLH).

The preferred ranges of the antigen and adjuvant doses are as follows:

Glycosylated MUC-1-G5: 0.1 to 30 μg;

Globo H, 0.1 to 100 μg;

GM2: 0.1 to 100 μg;

Le^y: 0.1 to 60 μg;

Tn(c): 0.1 to 100 μg;

APPENDIX B

Results

ELISA

	Globo H				GM2				Le^y			
	IgG		IgM		IgG		IgM		IgG		IgM	
Mouse #	presera	post 3rd	presera	post 3rd	presera	post 3rd	presera	post 3rd	presera	post 3rd	presera	post 3rd
1	0	40	120	1,080	40	40	40	80	0	40	80	640
2	0	80	40	3,240	40	40	40	80	0	40	80	80
4	0	360	120	3,240	0	0	0	80	0	40	40	640
5	0	40	0	3,240	0	40	0	80	0	160	40	320
+control	VK9 1:25,600						αGM2 >>>1:1,000,000		BR96(1 ug/ul) 1:3,200			

	MUC1G5				Tf-'HSA				Tn-'HSA			
	IgG		IgM		IgG		IgM		IgG		IgM	
Mouse #	presera	post 3rd	presera	post 3rd	presera	post 3rd	presera	post 3rd	presera	post 3rd	presera	post 3rd
1	0	10,240	0	80	0	640	0	80	0	1,280	0	80
2	0	20,480	0	320	0	640	0	160	0	640	0	5,120
4	0	5,120	0	40	0	5,120	0	160	0	10,240	0	640
5	0	10,240	0	160	0	10,240	0	160	0	10,240	40	640
+control							49H.8 1:1,600				aTn 1:25,600	

FACS

	MCF7						LSC					
	IgG		IgM				IgG		IgM			
Mouse #	presera	post 3rd	presera	post 3rd	+controls		presera	post 3rd	presera	post 3rd	+controls	
1	10.02%	95.76%	11.52%	89.79%	VK9	1.13%	10.11%	42.28%	9.63%	58.23%	VK9	0.95%
2	10.49%	95.30%	10.48%	95.61%	BR96	97.11%	9.60%	28.88%	10.75%	93.47%	BR96	94.85%
4	9.78%	94.71%	11.36%	95.96%	HMFG1	62.31%	9.93%	27.09%	11.12%	96.28%	HMFG1	1.19%
5	9.78%	95.48%	9.86%	94.49%	αTn AB	78.62%	10.59%	23.46%	10.16%	93.23%	αTn Ab	57.63%
2^0Ab alone		1.28%		1.15%	αGM2 Ab	94.91%		1.18%		1.14%	αGM2 Ab	63.94%
					49H.8	0.14%					49H.8	0.26%

sTn(c): 0.1 to 100 μg;
TF(c): 0.1 to 30 μg;
QS-21: 25-200 μg;
GPI-0100:1-25 mg.

Example 1

Phase I Clinical Trial Protocol Using the Heptavalent Vaccine

Example 2

Heptavalent Vaccine Immunogenicity Trial in Mice

1. Methods
2. Results

Example 1

Pilot Phase I Trial in Patients with Epithelial Ovarian, Fallopian Tube, or Peritoneal Cancer with a Polyvalent Vaccine-KLH Conjugate+QS-21

1.0 PROTOCOL SUMMARY
2.0 OBJECTIVE
3.0 BACKGROUND AND RATIONALE
4.0 VACCINE PREPARATION
5.0 TREATMENT SCHEDULE AND DOSE
6.0 EVALUATION DURING STUDY
7.0 BIOSTATISTICAL CONSIDERATIONS (Endpoints)
8.0 BIBLIOGRAPHY

1.0 Protocol Summary and Program Plan

Patients with epithelial ovarian, fallopian tube, or peritoneal cancer who receive surgical cytoreduction and platinum/taxane containing chemotherapy have a significant chance of entering complete clinical remission but unfortunately approximately 70% will eventually relapse. These patients in clinical remission have minimal residual disease, and are excellent candidates in which to evaluate novel consolidation strategies in an attempt to improve outcome. This pilot polyvalent protocol represents the culmination of a series of monovalent phase I vaccine trials at the center demonstrating the immunogenicity of the various component antigens. It represents the transition between the phase I monovalent trial program in second remission, to the planned development of larger trials designed to evaluate efficacy. Immunization with the individual antigens selected for this vaccine has been consistently immunogenic in the majority of patients. No confirmed systemic toxicity has occurred related to vaccine administration. It is expected that the immunogenicity will remain unchanged, and that no systemic toxicity will occur with polyvalent vaccine administration. Eligible patients for this pilot trial are those patients initially with stage II-IV disease in complete clinical remission following primary therapy, or following relapse and re-induction to remission with additional chemotherapy. In this trial, patients will receive an antigen defined vaccine with the following ganglioside components: a) GM2, b) Globo-H; the blood group related antigens: c) TF(c), d) s-Tn (c), e) Tn (c) f) Lewis-Y; and g) the protein antigen MUC-1-G5 (glycosylated). The primary endpoints of this pilot study are safety, and confirmation of continued immunogenicity. The secondary endpoint will be to characterize the nature and duration of the antibody response.

2.0 Objectives

2.1 The primary endpoints of this pilot study are to determine the safety of polyvalent vaccine administration, and continued immunogenicity in patients prior to conducting a large, randomized study.

2. 2.2 The Secondary Endpoint is to Further Characterize the Nature and Duration of the Antibody Response Generated by the Polyvalent Vaccine (ELISA and Facs)

3.0 Background and Rationale

3.1 Disease Background and Suitability for Treatment

In 1999, approximately 22,500 new cases of ovarian cancer were diagnosed, and it is estimated that 14,500 women died of the disease. Seventy-five percent of patients with ovarian cancer will have spread beyond the ovary at diagnosis. Standard primary treatment consists of cytoreductive surgery followed by a platinum and paclitaxel containing chemotherapy regimen.[1] Many patients have no clinically measurable disease at the end of primary treatment. A review of second-look laparotomy, however, indicates that less than 50% of patients are actually free of disease.[2] Furthermore, nearly half of patients with a negative second look procedure are destined to relapse and require additional treatment.[3,4] Overall, only approximately 30% of patients remain disease free with currently available treatment. Given the minimal disease burden at the completion of primary therapy, these patients are ideal candidates in which to evaluate immune modulating strategies.

3.2 Rationale for Polyvalent Vaccines Designed Primarily for Antibody Production

Varied data exists in solid tumors to support the development of immune directed therapy. Studies have emerged in patients with melanoma which demonstrate that naturally acquired[5,6], or actively induced[7,8] antibodies may improve outcome. In a large clinical trial reported by Reithmuller et al., 189 patients with resected Dukes C colon carcinoma were randomized to receive observation versus postoperative treatment with murine antibody CO17-1A that recognizes the KSA antigen. Toxic effects were limited to infrequent constitutional symptoms. At median follow-up of five years, the death rate was 36% in the treated group versus 51% in the observed group. The advantage of treatment was demonstrated in univariate (p=0.051) and multivariate (p=0.043) analysis when controlling for other known prognostic factors. [9]

The basis for cancer vaccines designed primarily for antibody induction are the many preclinical models demonstrating the ability of passively administered or actively induced antibodies to prevent tumor recurrence[10], the increasing number of clinical trials where passively administered monoclonal antibodies have demonstrated clinical efficacy, and the correlation of antibodies, naturally acquired or vaccine induced, with improved prognosis in several different clinical settings.[6]

EL4 lymphoma naturally expresses GD2 ganglioside, which is recognized by monoclonal antibody 3F8. Vaccines containing GD2 covalently conjugated to KLH and mixed with immunological adjuvant QS21 are the optimal approach to vaccination against GD2. Relatively higher levels of antibody administered two or four days after intravenous tumor challenge or moderate titers induced by vaccine that were present by day two or four after tumor challenge were able to eradicate disease in most mice. If antibody administration was deferred until day seven or ten, little or no benefit could be demonstrated. If the number of cells in the EL4 challenge was decreased, giving a longer window of opportunity, the vaccinations could be initiated after tumor challenge and good protection seen.[7] These results are consistent with the

need to initiate immunization with vaccines inducing antibodies in the adjuvant setting, when the targets are circulating tumor cells and micrometastases. Patients with ovarian cancer in first remission meet these criteria, and unfortunately have a high "event rate" (ie. 80% will relapse) allowing for the rapid assessment of the efficacy of this approach.

The basis for the inventors' emphasis on polyvalent vaccines is tumor cell heterogeneity, heterogeneity of the human immune response and the correlation between overall antibody titer against tumor cells and effector mechanisms such as complement mediated cytotoxicity (CDC) or antibody dependent cell mediated cytotoxicity (ADCC). For example, using a series of 14 tumor cell lines and monoclonal antibodies (mAbs) against 3 gangliosides, investigators at MSKCC have shown that significant cell surface reactivity analyzed by flow cytometry and CDC increased from 2-8 of the cell lines using one of three mAbs to 13-14 of the cell lines when the 3 mAbs were pooled. The median CDC increased 4 fold with the pool of mAbs compared to the best single mAbs.[11] Cancers of the ovary express a rich array of cell surface antigens making them especially suitable targets for polyvalent vaccines.

Cell surface antigens (especially carbohydrate cell surface antigens) have proven to be unexpectedly potent targets for immune recognition and attack of human cancers. Many of the more tumor-restricted monoclonal antibodies derived by immunization of mice with human tumor cells have been found to be directed against carbohydrate antigens expressed at the cell surface[12] Immunization against carbohydrate antigens results generally in an antibody response (see references for dissenting views), which is primarily IgM.[13-15] These antibodies are known to induce CDC, inflammation, and phagocytosis of tumor cells by the reticulo-endothelial system (opsonization).[16] Immunization against cell surface protein antigens can induce a variety of B and T lymphocyte responses. The T lymphocyte responses are difficult to quantify in the context of vaccination trials and are not the focus of this proposal. The antibody responses against protein antigens contain IgM and IgG, both of which can induce complement activation (with regard to IgG depending on the subclass, IgG1 and IgG3 being optimal). IgG antibodies of these subclasses can also induce ADCC.

Antibodies are the primary mechanism for active elimination of circulating pathogens from the bloodstream. They are ideally suited for eradication of free tumor cells and systemic or intraperitoneal micrometastases and they have accomplished this as described above in a variety of preclinical mouse experiments (reviewed in references).[10,7] In adjuvant immunization trials, the primary targets are individual tumor cells or early micrometastases which may persist for long periods after apparent resection of all residual tumor.[17] After surgery and completion of chemotherapy is the ideal time for immune intervention, and in particular for administration of cancer vaccines aimed at instructing the immune system to identify and kill the few remaining cancer cells. If antibodies of sufficient titer can be induced against tumor antigens to eliminate tumor cells from the blood and lymphatic systems, aggressive local therapies, including surgery, radiation therapy and intralesional treatments might result in long term control of even metastatic cancers.

3.3 Preliminary Studies for the Antigens

GM2 Vaccines:

Investigators at the center have been refining the ability to induce antibodies against GM2 in melanoma patients for fifteen years, since first demonstrating that vaccines containing purified GM2 could be more immunogenic than vaccines containing tumor cells expressing GM2.[18] Initially GM2 adherent to BCG was selected as optimal, inducing IgM antibodies in 85% of patients. This was the basis for a randomized trial comparing immunization with BCG to immunization with GM2/BCG in 122 patients with AJCC Stage 3 melanoma.[8] The IgM antibodies had a median titer of $1/160$ and were short lived (8-12 weeks). IgG antibody induction was rare. Antibody titers have been maintained for over three years by administration of repeated booster immunizations at 3-4 month intervals. When comparing patients as randomized in this trial, no statistically significant difference on overall or disease free survival was seen. Pre-existing GM2 antibodies were seen in 5 patients in the control group, as opposed to one in the GM2 treated group which may have blunted the treatment result. The association between better outcome and the presence of GM2 antibodies was seen (8).

TF, Tn and sTn Vaccines:

Patients with various epithelial cancers have been immunized with unclustered TF-KLH and sTn-KLH conjugate vaccines plus various adjuvants:[19] High titer IgM and IgG antibodies against TF and sTn antigens have resulted, but we found that the majority of the reactivity detected in sera from immunized mice and patients was against antigenic epitopes present on synthetic constructs which were not present on naturally expressed mucins.[20] Based on previous studies with Tn antigen,[21] Kurosaka and Nakada et al. hypothesized that MLS102, a monoclonal antibody against sTn, might preferentially recognize clusters (C) of sTn. In studies at MSKCC with monoclonal antibody B72.3 and with sera raised against TF-KLH and sTn-KLH conjugate vaccines in mice and in patients resulted in the same conclusion.[20,22] The availability of synthetic TF, Tn and sTn clusters consisting of 3 epitopes covalently linked to 3 consecutive serines or threonines has permitted investigators at MSKCC to prove this hypothesis. In both direct tests and inhibition assays, B72.3 recognized sTn clusters exclusively, and sera from mice immunized with sTn (C)-KLH reacted strongly with both natural mucins and tumor cells expressing sTn.[22] Based on this background, we initiated trials with the TF(C)-KLH, Tn(C)-KLH and sTn(C)-KLH conjugate vaccines in patients with breast cancer. Antibodies of relevant high titer specificity, including against OSM or PSM and cancer cells expressing TF, Tn or sTn, have been induced for the first time in the inventors' experience. Based on these results confirming the importance of clustered epitopes and defining their relevant immunogenicity, we are including these clustered antigens in the polyvalent vaccine against ovarian cancer.

Le^y and Globo H Vaccines:

The development of Le^y and Globo H vaccines was previously limited by the lack of sufficient quantities of antigen for vaccine construction and testing. Over the last four years, Dr. Danishefsky has successfully synthesized both antigens.[23,24] Investigators at MSKCC have immunized groups of mice with Globo H-ceramide plus or minus adjuvants QS-21 and Salmonella minnesota mutant R595, and with Globo H covalently attached to KLH or BSA plus immunological adjuvant QS-21. The highest antibody titers against both synthetic antigen and MCF7 cells expressing Globo H were induced by the Globo H-KLH plus QS-21 vaccine.[23,24] The antibody titer induced against synthetic Globo H was $1/120{,}000$ by ELISA, the titer induced against MCF7 was $1/320$, and potent complement mediated cytotoxicity was seen as well. Le^y-BSA and Le^y-KLH vaccines have also been tested in the mouse. High titer antibody responses have resulted against the synthetic epitope of Le^y and against tumor cells expressing Le^y in the majority of mice immunized.[25] Based on these results, monovalent phase I clinical trials with Globo H-KLH plus QS-21 and Le^y-KLH plus QS21 have been initiated in

patients with breast, prostate or ovary cancer. Antibodies against the purified antigens and against tumor cells expressing these antigens were induced in most patients and the manuscript was recently published for the latter.[26,27]

MUC1 and MUC2 Vaccines:

Investigators at MSKCC have immunized mice with MUC1-KLH and MUC2-KLH, plus QS-21, and seen induction of consistent high titer IgM and IgG antibodies against MUC1 and MUC2 and human cell lines expressing MUC1 and MUC2, as well as protection from a syngeneic mouse breast cancer expressing human MUC1 as a consequence of gene transduction. Mice were also immunized with vaccines containing MUC1 peptides of various lengths conjugated to KLH by one of three methods or not, and mixed with QS-21 or BCG. MUC1 containing 30 amino acids or more, conjugated to KLH with an MBS bifunctional linker and mixed with immunological adjuvant QS-21 induced the highest titer antibodies.[28] Based on these studies in the mouse, a trial was initiated and completed a trial with this MUC1-KLH plus QS-21 vaccine in breast cancer patients who were free of detectable breast cancer after resection of all known disease. Nine patients were treated with a 31 amino acid MUC1 peptide with cysteine at one end for conjugation to KLH and the immune dominant epitope -APDTRPA at the other end.29 No patient had detectable MUC1 serological reactivity by ELISA or FACS prior to immunization. The results are summarized below in table below. Reactivity against MUC1 and tumor cells expressing MUC1 was seen in most patients. A separate group of patients were immunized with MUC2-KLH plus QS21. Analysis of this trial is not yet complete, but the results to date are also summarized in the table below.

The inventors have been unable to demonstrate T-lymphocyte proliferation, interferon γ and IL4 release by ELISPOT assays, CTL activity or positive DTH responses after vaccination with MUC1 or MUC2. The proliferation assays were particularly focussed on in the MUC1 trials. Patients had leukophoresis pre and post vaccination, providing ample lymphocytes for study. After 2 years of steady endeavor, there has been no clear evidence of augmented reactivity against MUC1 peptides of various lengths or, in HLA A2 positive patients against heteroclytic MUC1 peptides with single amino acid changes that increased binding to HLA A2. (personal communication, P. O. Livingston) Pre and post vaccination PBLs from the first 6 patients vaccinated with MUC1 were also sent to the laboratory of Dr. Olivera Finn for CTL precursor frequency analysis. No increase in frequency was seen. Over all, the major difference between results from MSKCC with the 31aa MUC1-KLH plus QS-21 vaccine and Dr. Finn's results with a 104aa MUC1 peptide plus BCG vaccine was that the former had a clearly demonstrable, consistent antibody response, which was reactive with tumor cells. Inhibition assays were performed to better understand this serologic response.[30] Much of the IgM response and nearly all of the IgG response were against the immune dominant epitope, APDTRPA, preferentially with RPA at the terminal position.

KSA Vaccines:

KSA has been prepared in the baculovirus system by Jenner Technologies (San Ramon, Calif.) and 10 mcg/patient has been provided for testing. Due to the small quantity of KSA available, following demonstration of relevant immunogenicity in mice, we treated groups of 9 patients with KSA plus QS21 or with KSA covalently linked to KLH by glutaraldehyde, plus QS21. In neither case was there significant induction of antibodies against KSA that had not been glutaraldehyde treated, or tumor cells expressing KSA. Consequently KSA will not be included in the polyvalent vaccine. The results of this and the other trials with KLH conjugate vaccines are summarized in the table below (personal data, P.O. Livingston).

Summary of Serological Results in Vaccinated Patients

	Median ELISA		IgG	Median FACS		Median	Median
Antigen	IgM	IgG	Subclass	IgM	IgG	IA	CDC
GM2	640	320	IgG1 + 3	+++	++	++	++
Globo H	640	40	IgG1 + 3	++	+	++	+
Lewis^y	80	0		++	+	+	+
Tn	1280	1280		++	−	+	−
STn	1280	160	IgG3	+++	−	+	−
TF	320	10			−	+	−
MUC1	1280	5120	IgG1 + 3	+	−	+	−
MUC2	2560	2560		pending			
KSA	40	160		−	−	−	−

Additional Variables:

Two additional variables have proven critical, the method of conjugation and the epitope ratio of antigen molecules per KLH molecule. The optimal conjugation approached has varied with the antigen. Gangliosides are best conjugated using ozone cleavage of the ceramide double bond and introducing an aldehyde group followed by coupling to aminolysyl groups of KLH by reductive amination. This approach was not as effective for conjugation of Tn, sTn and TF clusters or Globo H to KLH where an M2C2H linker arm has proved most efficient[31] or for MUC1 or MUC2 where an MBS linker group was optimal.[28]

The impact of dose and schedule of vaccine administration on antibody response to GM2 vaccines in melanoma patients has also been explored. Immunization 4 times at weekly intervals or biweekly intervals followed by booster immunizations twice at 2-3 month intervals was compared to 6 immunizations at monthly intervals. Initial immunizations at weekly or biweekly intervals resulted in comparable high titers (with high titers occurring slightly sooner at weekly intervals), but remarkably the monthly immunizations resulted in far weaker or undetectable antibody responses in the 6 patients vaccinated.[32] GM2-KLH plus QS-21 vaccines prepared at MSKCC and at Progenics Pharmaceuticals have each been tested in dose finding studies such as those proposed in this application. In both cases GM2 doses of 3 ug or less resulted in lower IgM titers and undetectable IgG titers in most patients. GM2 doses of 10, 30 and 100 ug gave comparable IgM and IgG titers.[33] Based an these studies, we have selected the initial weekly schedule of 3-4 immunizations followed by booster immunizations every three months, and the doses for use in the randomized Phase III trial.

3.4 Potential Toxicity of Vaccination

The expected safety of the vaccine is based on the safety of vaccination with the individual components. Clinical experience is growing in clinical trials with vaccine induced antibody responses against each of the included antigens. Antigen expression at secretory borders in these trials, where the majority is located, has induced neither immunological tolerance nor symptomatic autoimmunity once antibodies are present, suggesting they are sequestered from the immune system. Nevertheless, a regular schedule of laboratory studies and physical examinations are designed to detect any abnormalities. This pilot trial will represent one of the first studies to confirm the safety of polyvalent vaccine administration in this setting.

3.5 General Immune Approaches

Various methods have been used to increase the immunogenicity of antigens, and in particular for inducing an IgG response. In preclinical laboratory studies, we have found the covalent attachment of antigen to keyhole limpet hemocyanin (KLH) to be most effective.[34] KLH is well tolerated, and has previously caused only mild inflammation at the vaccine injection site. Attachment of KLH may be accomplished by a variety of cross-linking methods. MBS (m-maleimidobenzoly-N-hydroxysuccinimide ester) is the best-known heterobifunctional reagent; and at neutral pH cross-links thiol groups with amino groups. The linkage proceeds via two separate reactions, thus limiting bonds between identical molecules. In addition to linking antigen to immunogenic carrier proteins, the titer of antibody induced may be further augmented with the use of appropriate immunological adjuvants. We have immunized groups of melanoma patients with vaccines either containing no adjuvant, or using DETOX, BCG or QS-21. QS-21 is a significantly more effective adjuvant than the others, producing significantly higher titer IgM and IgG antibodies. It is a saponin derivative extracted from the bark of the South American tree *Quillaja saponaria* Molina. The monosaccharide composition, molecular weight, adjuvant effect and toxicity for a series of these saponins have been described.[35-36] It has also proven to be non-toxic and effective at augmenting the immunogenicity of an FeLV subunit vaccine in cats[37] and an HIV-1 recombinant vaccine in Rhesus monkeys. A phase I trial demonstrating the safety and suggesting the efficacy of a 100 ug QS-21 dose in patients treated with GM2-KLH vaccines has been reported. The only adverse events reported were minimal flu-like symptoms, and mild discomfort at the injection site.[38] Thus, conjugation with KLH and the addition of QS-21 have become standard approaches for vaccine construction at MSKCC has proven optimal for antibody induction against a variety of gangliosides, MUC1, MUC2, KSA, Tn, sTn, TF, Le^y and Globo H in the mouse and in humans.

3.6 Distribution of the Antigens Studied:

In general, the antigens contained in this vaccine are expressed on ovarian cancer cells with high frequency. Recent studies at MSKCC have characterized this distribution in a variety of tumor types including ovarian cancer using immunohistochemical staining. A variety of tumor specimens were used in each tumor type, and it was required that 50% or more cells be positive in order to consider the antigen present. The presence of these antigens on the tumor specimens tested in ovarian cancer was: GM2 (100%), GLOBO-H (60%), MUC 1 (100%), sTn (60%), TF (100%), Le Y (80%).[39-41]

3.7 Rational for the Inventors' Approach:

The inventors' hypothesis is that induction of an antibody response against several cell surface antigens on ovarian cancer cells with a polyvalent conjugate vaccine will result in eradication of free tumor antigen, circulating tumor cells and micrometastases. The polyvalent nature of the vaccine and antibody response is important to eliminate escape by tumor cells that fail to express any one or two of the antigens, and to increase the number of antibodies reacting against each cell. It is expected that the inventors' vaccine will prove consistently immunogenic against five or six of the ovarian cancer cell surface antigens described above, and that it will prove nontoxic. Whether immunization with this polyvalent vaccine in high-risk ovarian cancer patients in the remission setting will result in prolonged disease-free and overall survival is the focus of subsequent studies to follow this pilot trial.

4.0 Vaccine Preparation

4.1 GM2 is provided by Progenics. GLOBO-H, Lewis-Y, TF(c), Tn (c), and sTn (c) are synthesized in the laboratory of Dr. Sam Danishefsky at the center. MUC-1 is produced at the core facility at MSKCC. For glycosylation, the MUC-1 was shipped to the University of Copenhagen, Glycobiology Group. The glycosylation was carried out by GalNAc transferase using UDP-N-GalNAc as substrate. The product was shipped back to MSKCC after purification by reverse phase HPLC.

4.2 QS-21 is obtained from Aquila Biopharmaceuticals in 100 mg vials as a white powder and is stored at −30 degrees Celsius. This is suspended initially in PBS as it is less soluble in normal saline and then final dilutions are made in normal saline. QS-21 is passed through a 0.22 micrometer filter immediately prior to use.

4.3 Conjugation to KLH is accomplished with three different conjugation procedures: direct amination (ozonolysis) for GM2, the M2C2H bifunctional linker group for Globo H and Le Y, and the MBS bifunctional linker group for the four mucin antigens.

4.4 The sterility of the conjugate is confirmed by passage through a 0.22 micrometer filter and it is stored in frozen normal saline at −30 to −80 degrees Celsius. 4.5 Vials are released for use following standard lot release testing (approximately five weeks).

5.0 Treatment Schedule and Dose

5.1 Vaccine Contains:

[GM2 (10 mcg)/TF(c)(3 mcg)/sTn(c)(3 mcg)/Globo-H(10 mcg)/MUC1-1-G5 (3 mcg)/Le^Y (10 mcg)/Tn(c)(3 mcg)]-KLH (≈400 mcg) with adjuvant QS21 (100 mcg)

5.2 Immunization Schedule

The vaccine will be administered at weekly intervals for 3 doses. This will be followed by a four week break and then a fourth vaccination. There will then be an eight week break and then a fifth vaccination, followed by additional immunizations every twelve weeks for 24 months total (as long as patient remains on study).

IMMUNIZATION SCHEDULE

	WEEK #				
	1	2	3	7	15
VACCINE #	1	2	3	4	5

5.3 Dose Administration and Modification

5.3.1 No dose escalation or dose modification will be performed. Systemic toxicity has not been seen with the previous vaccine studies. Systemic toxicity>grade II (with the exception of fever without infection) thought related to vaccination would result in removal of the patient from study and suspension of the protocol pending investigation.

5.3.2 The preparation will be administered subcutaneously to a site in the shoulders, buttocks, or thighs. It will be administered in one syringe, and will be supplied in approximately 1 cc total volume.

6.0 Evaluation During Study

6.1 Immune Function (Summarized in Table)

6.1.1 Antibody Response:

Peripheral blood (20-30 ml) will be drawn according to the schedule in table 8.3 with the exception of week 0, 7, and 9 at which time 50-60 ml will be collected for antibody testing. Thereafter, blood will be drawn at 12-week intervals as the patients return for booster immunizations. Antibodies against

various antigens will be studies by ELISA, and against human tumor cell lines by FACS when appropriate.

6.2 Clinical and Laboratory Assessment:

6.2.1 Clinical and Laboratory Assessment Schedule:

The clinical and laboratory assessment schedule is outlined in Section 8.3 and includes parameters to assess the safety of the vaccine, as well as evaluate for signs of disease recurrence and progression. Abnormal findings will be evaluated per standard medical practice, and the abnormality will be classified as related to treatment, disease progression, or neither.

6.2.3 Extent of Disease Evaluation:

All patients are by definition without clinical or radiographic evidence of disease at protocol entry.

6.2.4 Radiographic Imaging

(CT abdomen and pelvis) will be obtained q 6 months while on study, or if any clinical symptoms/examination findings warrant further evaluation, or if serum CA-125 rises to >70 (per time to treatment failure criteria), confirmed by repeat value; or at any time at the discretion of the investigator.

6.2.5 Length of Follow-Up:

The primary endpoint of the study in this pilot trial is safety. Patients will be followed for the duration of the study, but based on previous trials, antibodies are generally present by the seventh week, and we will proceed with the proposal for additional studies to evaluate efficacy if no systemic toxicity is seen in any patient at the ninth week assessment. An additional 8-12 weeks would be required for processing before patients could be enrolled on the polyvalent study, allowing ample time for follow-up of the pilot trial. Patients will be followed until time to treatment failure, or until all vaccinations are completed (maximum 24 months).

6.3 Summary of Evaluation:

SUMMARY OF CLINICAL, LABORATORY, AND IMMUNE ASSESSMENTS

	week #										
	0[a]	1	2	3	5	7	9	13	15	17	27[c]
vaccine #		1	2	3		4			5		V
Office Visit	*	*	*	*	*	*	*	*	*	*	*
Hx and PE	*		*	*		*	*	*	*	*	*
CA-125	*				*		*	*	*		*
CBC, diff	*		*	*	*	*	*	*	*	*	*
Hepatic profile + creatinine	*		*	*	*	*	*	*	*	*	*
Amylase	*		*	*	*	*	*	*	*	*	*
Urinalysis	*			*		*			*		*
PT	*										
Stool guaiac[b]	*					*			*		*
TSH	*								*		
Immune[d] bloods		*				*	*	*	*	*	*

[a]pretreatment evaluation, within 3 weeks

[b]stool guaiac may be obtained by digital exam or cards collected by patient

[c]following week 27, visits + laboratory studies + immunization q 3 months, CT scan q 6 months while on study

[d]immune bloods routinely consist of 20-30 ml collected in 3 red top tubes. In order to obtain sufficient serum to evaluate for multiple antibodies, 6 tubes will be collected instead of 3 at the pre-vaccination visit; and at week 7 and 9 only.

7.0 Biostatistical Considerations

Endpoints:

The Primary Endpoints of this Pilot Trial are Safety and Confirmation of Immunogenicity in the Polyvalent Setting.

No systemic toxicity has occurred with the administration of monovalent vaccines at the center. Toxicity is not expected with this preparation. Following this pilot, additional studies with efficacy as the endpoint will be proposed. This pilot trial would be suspended pending investigation for any systemic toxicity thought related to vaccine in any patient. The same criteria for immunogenicity will be used as that of the individual pilot trials: patients must have IgM titer ≥1:80, or a four fold increase in prevailing antibody titer if present at baseline. Nine patients will be accrued, and ≥5 of 9 patients should meet these criteria for three or more antigens in order to proceed with this construct in additional studies. In prior trials, antibodies are generally present by completion of the fourth vaccination (week 7). If no systemic toxicity is seen by the week 9 assessment in these patients, we will proceed with proposals for phase II studies with efficacy endpoints.

While not the endpoint of this pilot, patients would be removed from study for relapse as defined below. Time to treatment failure will be simply defined based on data from Rustin et al.[42] Treatment failure can be characterized by 1) physical examination evidence of disease recurrence, radiographic evidence of disease recurrence (biopsy will be performed at the discretion of the principal investigator but is not required); or 3) CA-125 elevation to twice the upper limits of normal (ie. ≥70), confirmed by a second sample also ≥70 U/ml. Time to treatment failure for biochemical relapse is recorded as the date of the first sample ≥70 U/ml.

The secondary endpoint of this pilot trial is to characterize the nature and duration of the immune response. Peripheral blood (20-30 ml) will be drawn as indicated in the table. Antibodies against the individual antigens will be studies by ELISA, and against the appropriate human tumor antigen FACS using human tumor cell lines expressing the respective antigen.

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Example 2

Heptavalent Vaccine Immunogenicity Trial 1N Mice

Methods

Serological Analyses

1. ELISA (Enzyme-Linked Immunosorbent Assay):

ELISA assays were performed as described below. Antigen in ethanol or in 0.1 M carbonate buffer (pH 11) were coated on ELISA plates at 0.2 μg/well for glycolipids and 0.1 μg/well for peptides. Serially diluted antiserum was added to each well and alkaline phosphatase-conjugated goat anti-mouse IgM or anti-mouse IgG was added at a dilution of 1:200

(Southern Biotechnology Associates, Inc, Birmingham, Ala.). Goat anti-mouse IgG and IgM conjugated with alkaline phosphatase obtained from Kierkegaard and Perry Labs, (Gaithersburg, Md.) were used as second antibodies. ELISA titer is defined as the highest dilution yielding an absorbance of 0.1 or greater over that of normal control mouse sera.

2. Cell Surface Reactivity Determined by FACS:

The cell surface reactivity of immune sera was tested on human MCF-7 and LSC cell lines. Single cell suspensions of 2×10^5 cells/tube were washed in PBS with 3% fetal calf serum (FCS) and 0.01M NaN_3 and incubated with 20 μl of 1:20 diluted sera or monoclonal antibody mAb for 30 min on ice. After two washes with 3% FCS in PBS, 20 μl of 1:15 diluted goat anti-mouse IgM or IgG-labeled with fluorescein-isothiocyanate (FITC, Southern Biotechnology Associates Inc. Birmingham, Ala.) was added, and the mixture incubated for 30 min. After a final wash, the positive population and mean fluorescence intensity of stained cells were differentiated using FACScan, Becton & Dickinson Immunocytometry, San Jose, Calif.

Immunizaton of mice with Heptavalent-KLH Conjugates plus ELISA
GPI100 0r QS21.
Mar. 19, 2001

ELISA plate coated with . . .	mice immunized with . . . / (Group#)		pre vacc. (IgG/IgM)	post vacc. (IgG/IgM)	Comments
GM2	GM2 (group #1)		0/0	0/0	
	GloboH (group #2)			0/0	
	LeY (group #3)			0/0	
	Muc1-G5 (group #4)			0/0	
	STn(c) (group #5)			0/0	
	TF(c) (group #6)			0/0	
	Tn(c) (group #7)			0/0	
Globo H	GM2 (group #1)				
	GloboH (group #2)		0/0	640/5120	
	LeY (group #3)				
	Muc1-G5 (group #4)				
	STn(c) (group #5)				
	TF(c) (group #6)				
	Tn(c) (group #7)				
LeY	GM2 (group #1)			0/0	
	GloboH (group #2)			0/0	
	LeY (group #3)		0/0	640/2560	
	Muc1-G5 (group #4)			0/0	
	STn(c) (group #5)			0/160	
	TF(c) (group #6)			0/40	
	Tn(c) (group #7)			0/40	
Muc1-G5	GM2 (group #1)			0/320	
	GloboH (group #2)			0/160	
	LeY (group #3)			0/160	
	Muc1-G5 (group #4)		0/0	2560+/1280	
	STn(c) (group #5)			40/80	
	TF(c) (group #6)			40/80	
	Tn(c) (group #7)			0/320	
	mAb.	C595		2560/	
		B55		/<5120+++	
STn(c)	GM2 (group #1)			0/0	
	GloboH (group #2)			0/0	
	LeY (group #3)			0/0	
	Muc1-G5 (group #4)			0/0	
	STn(c) (group #5)		0/0	5120/320	
	TF(c) (group #6)			0/0	
	Tn(c) (group #7)			0/0	
	mAb.	cc49		1280/0	
Tf(c)	GM2 (group #1)			0/40	
	GloboH (group #2)			40/80	
	LeY (group #3)			40/40	
	Muc1-G5 (group #4)			5120+/160	
	STn(c) (group #5)			5120+/320	
	TF(c) (group #6)		0/0	5120+/320	
	Tn(c) (group #7)			5120+/640	
	mAb.	JAA-F11		0/	
		A78-6/A7		/640	
Tn(c)	GM2 (group #1)			0/0	
	GloboH (group #2)			0/0	
	LeY (group #3)			0/0	
	Muc1-G5 (group #4)			5120+/160	
	STn(c) (group #5)			5120+/320	
	TF(c) (group #6)			5120+/320	
	Tn(c) (group #7)		0/0	5120+/2560	
	mAb.	5F4		/5120++	
		HB-Tn1		/5120	

Immunization of mice with Heptavalent-KLH Conjugates* plus GPI-100 or QS-2

Group #	Antigen	Mice #	ELISA (IgG/IgM) Pre-vacc.	ELISA (IgG/IgM) Post-vacc	FACS (IgG/IgM) Pre-vacc.	FACS (IgG/IgM) Post-vacc.	Comments
8. Heptavalent-KLH + 100 ug GPI-100 (Batch J) (200 ul/mouse)	Tn(c)	1	0/0	5120+/160			
		2	0/0	5120/160			
		3	0/0	5120/160			
		4	0/0	5120++/640			
		5	0/40	5120+/320			
	Tf(c)	1	0/0	5120/160			
		2	0/0	5120/160			
		3	0/0	5120/160			
		4	0/0	5120+/320			
		5	0/40	5120+/640			
	sTN(c)	1	0/0	5120/640			
		2	0/0	1280/640			
		3	0/0	5120/640			
		4	0/0	5120/320			
		5	0/0	1280/320			
	MUC1-1G5	1	0/0	5120+/0			
		2	0/0	5120+/80			
		3	0/0	5120+++/160			
		4	0/0	5120++/40			
		5	0/0	5120+++/80			
	Ley	1	0/0	0/40			
		2	0/0	320/640			
		3	0/0	0/160			
		4	0/0	80/40			
		5	0/0	0/640			
	Globo H	1	0/80	40/640			
		2	0/80	0/640			
		3	0/80	0/640			
		4	0/160	40/1280			
		5	0/160	0/2560			
	GM2	1	0/0	0/40			
		2	0/0	0/0			
		3	0/0	0/0			
		4	0/0	0/0			
		5	0/0	0/0			

Immunization of mice with Heptavalent-KLH Conjugates* plus GPI-100 or QS-21.
Apr. 2, 2001

Group #	Antigen	Mice #	ELISA (IgG/IgM) Pre-vacc.	ELISA (IgG/IgM) Post-vacc	FACS (IgG/IgM) Pre-vacc.	FACS (IgG/IgM) Post-vacc.	Comments
9. Heptavalent-KLH + 100 ug GPI-100 (Batch J) (200 ul/mouse)	Tn(c)	1	0/0	640/40			
		2	0/0	640/0			
		3	0/0	5120/40			
		4	0/0	5120+/0			
		5	0/0	1280/40			
	Tf(c)	1	0/0	1280/80			
		2	0/0	1280/40			
		3	0/0	5120+/40			
		4	0/0	5120++/40			
		5	0/0	2560/40			
	sTN(c)	1	0/0	320/80			
		2	0/0	320/160			
		3	0/0	640/160			
		4	0/0	640/80			
		5	0/0	80/320			
	MUC1-1G5	1	0/0	2560/0			
		2	0/0	640/0			
		3	0/0	640/0			
		4	0/0	640/0			
		5	0/0	2560/80			
	Ley	1	0/0	0/0			
		2	0/0	0/80			
		3	0/0	0/0			
		4	0/0	0/0			
		5	0/0	160/1280			
	Globo H	1	0/80	0/160			
		2	0/80	0/320			
		3	0/80	0/160			
		4	0/80	0/320			
		5	0/80	40/320			

-continued

	GM2	1	0/0	0/0
		2	0/0	0/0
		3	0/0	0/0
		4	0/0	0/0
		5	0/0	0/0
10. Heptavalent-	Tn(c)	1	0/0	5120++/320
KLH +		2	0/0	5120/320
100 ug GPI-		3	0/40	5120+/1280
100 + polysorbate 80		4	0/0	5120+/640
(200 ul/mouse)		5	0/0	2560/320
	Tf(c)	1	0/0	5120+/320
		2	0/0	5120+/160
		3	0/0	5120+/640
		4	0/0	5120+/640
		5	0/0	5120+/320
	sTN(c)	1	0/0	320/640
		2	0/0	320/1280
		3	0/0	1280/1280
		4	0/0	640/40
		5	0/0	320/320
	MUC1-	1	0/0	1280/40
	1G5	2	0/0	5120/80
		3	0/0	5120/160
		4	0/0	2560/160
		5	0/0	5120/40
	Ley	1	0/0	0//0
		2	0/0	0/0
		3	0/0	0/640
		4	0/0	0/640
		5	0/0	0/80
	Globo H	1	0/40	80/640
		2	0/80	40/2560
		3	0/320	0/2560
		4	0/160	0/2560
		5	0/80	0/640
	GM2	1	0/0	0/0
		2	0/0	0/0
		3	0/0	0/0
		4	0/0	0/0
		5	0/0	0/0
11. Heptavalent-	Tn(c)	1	0/0	5120/160
KLH + +		2	0/0	5120+/160
10 ug QS-21		3	0/40	5120+++/160
(200 ul/mouse)		4	0/80	2560/320
		5	0/0	5120+/80
	Tf(c)	1	0/40	5120+/320
		2	0/0	5120++/640
		3	0/160	5120++640
		4	0/320	2560/640
		5	0/40	5120++/320
	sTN(c)	1	0/0	40/40
		2	0/0	2560/640
		3	0/0	5120/160
		4	0/0	1280/80
		5	0/0	5120/640
	MUC1-	1	0/0	1280/160
	1G5	2	0/0	2560/80
		3	0/0	5120/80
		4	0/0	2560/320
		5	0/0	5120++/80
	Ley	1	0/0	0//0
		2	0/0	0/0
		3	0/0	0/0
		4	0/0	0/0
		5	0/0	40/40
	Globo H	1	0/80	40/320
		2	0/80	80/320
		3	0/80	40/640
		4	40/160	0/640
		5	0/320	0/2560
	GM2	1	0/0	0/0
		2	0/0	0/0
		3	0/0	0/0
		4	0/0	0/0
		5	0/0	0/0

-continued

12. Heptavalent-KLH + 3 ug ER-803022 (200 ul/mouse)	Tn(c)	1	0/40	2560/160
		2	0/0	640/160
		3	0/40	320/320
		4	0/0	160/160
		5	0/0	2560/160
	Tf(c)	1	0/0	2560/40
		2	0/0	2560/40
		3	0/0	640/160
		4	0/0	320/80
		5	0/0	2560/0
	sTN(c)	1	0/0	0/40
		2	0/0	40/80
		3	40/40	80/160
		4	0/0	160/160
		5	0/0	40/0
	MUC1-1G5	1	0/0	80/0
		2	0/0	80/0
		3	0/0	80/40
		4	0/0	40/80
		5	0/0	40/0
	Ley	1	0/0	0/0
		2	0/0	0/0
		3	0/0	0/0
		4	0/0	0/80
		5	0/0	0/0
	Globo H	1	0/80	0/320
		2	0/160	40/1280
		3	0/320	0/320
		4	80/160	160/1280
		5	40/160	320/640
	GM2	1	0/0	0/0
		2	0/0	0/0
		3	0/0	0/0
		4	0/0	0/0
		5	0/0	0/0
13. Heptavalent-KLH + 10 ug ER-803732 (200 ul/mouse)	Tn(c)	1	0/40	40/320
		2	0/0	40/40
		3	0/0	320/160
		4	0/40	160/160
		5	0/40	160/80
	Tf(c)	1	0/0	160/160
		2	0/0	320/40
		3	0/40	640/160
		4	0/40	320/160
		5	0/0	320/160
	sTN(c)	1	0/0	40/80
		2	0/0	0/80
		3	0/0	0/320
		4	0/160	40/320
		5	0/40	40/160
	MUC1-1G5	1	0/0	640/40
		2	0/0	0/40
		3	0/0	40/80
		4	0/40	80/160
		5	0/0	160/80
	Ley	1	0/0	0/0
		2	0/0	0/0
		3	0/40	40/320
		4	0/0	0/40
		5	0/0	0/80
	Globo H	1	40/80	80/160
		2	0/160	40/320
		3	0/40	80/320
		4	0/80	0/160
		5	80/160	160/320
	GM2	1	0/0	0/0
		2	0/0	0/0
		3	0/0	0/0
		4	0/0	0/0
		5	0/0	0/0
14. 30 ug KLH + 100 ug GPI-100 (100 ul/mouse)	Tn(c)	1	0/0	0/160
		2	0/0	0/40
		3	0/0	0/0
		4	0/0	0/160
		5	0/40	0/640

-continued

Tf(c)	1	0/40	0/320
	2	0/40	0/160
	3	0/0	0/80
	4	0/0	0/320
	5	0/160	40/640
sTN(c)	1	0/0	0/80
	2	0/0	0/40
	3	0/0	0/0
	4	0/0	0/320
	5	0/0	0/80
MUC1-1G5	1	40/40	0/160
	2	0/40	0/80
	3	0/0	0/80
	4	0/0	0/320
	5	0/0	0/320
Ley	1	0/0	0/40
	2	0/0	0/0
	3	0/0	0/0
	4	0/0	0/0
	5	0/0	0/0
Globo H	1	0/320	40/1280
	2	0/320	0/640
	3	0/80	0/1280
	4	0/160	0/640
	5	0/160	40/640
GM2	1	0/0	0/160
	2	0/0	0/40
	3	0/0	0/0
	4	0/0	0/320
	5	0/0	0/160
KLH	1	0/0	5120++/320
	2	0/40	5120++/160
	3	0/0	5120++/320
	4	0/0	5120++/640
	5	0/40	5120++/320

Immunization of mice with Heptavalent-KLH Conjugates* plus GPI-100 or QS-21.

FACS		% positive cells					
Group # (mice		MCF-7 (IgG/IgM)			LSC (IgG/IgM)		
immunized with . . .)	Mice #	Pre-vacc.	Post-vacc.	1:200 dilut.	Pre-vacc.	Post-vacc.	1:200 dilut.
1. 3 ug GM2-KLH +	1	11.79/10.68	21.45/11.79	15.73/0.38	10.90/9.58	6.91/2.93	6.56/0.14
100 ug GPI-100	2	10.23/10.69	4.80/1.49	2.34/0.0	10.90/10.45	5.99/3.45	1.45/0.59
(100 ul/mice)	3	9.50/10.74	6.95/15.79	0.99/0.21	11.29/11.11	7.79/1.53	6.41/0.23
	4	9.81/9.68	3.41/12.63	0.99/2.06	9.76/11.06	6.94/1.75	3.42/0.02
	5	10.81/10.42	10.43/3.15	12.29/0.11	10.13/10.76	4.40/1.67	0.40/0.10
2. 3 ug GloboH-KLH +	1	10.63/9.93	5.79/17.26	6.60/0.19	9.87/10.99	7.08/2.77	9.55/0.25
100 ug GPI100	2	10.08/10.67	16.60/27.63	10.54/6.75	10.0/10.41	8.9/3.43	1.29/0.52
(100 ul/mice)	3	11.03/10.09	27.21/40.86	27.58/2.30	10.42/10.61	11.56/1.15	7.60/0.02
	4	10.16/11.36	1.92/13.77	2.30/1.03	10.57/11.04	5.29/2.43	3.81/0.24
	5	11.94/10.82	24.61/12.97	20.32/3.62	10.79/10.72	10.58/2.54	1.25/0.20
3. 3ug Ley-KLH +	1	11.01/9.97	85.01/43.57	41.26/1.73	10.22/10.10	89.17/44.27	13.40/1.87
100 ug GPI-100	2	9.95/9.69	47.75/78.17	4.86/3.43	10.49/8.39	91.69/79.9	7.64/7.04
(100 ul/mice)	3	10.41/10.58	69.55/10.95	20.53/0.12	11.30/9.47	95.57/21.25	24.67/0.16
	4	11.93/10.03	91.08/0.76	21.29/0.0	10.96/9.60	81.5/6.24	9.08/0.38
	5	12.46/11.18	42.94/36.67	13.66/1.87	10.98/10.61	12.74/52.16	1.05/1.87
4. 3 ug Muc-1G-5-	1	11.33/10.94	98.12/9.52	97.98/0.93	9.50/9.89	4.83/1.10	2.29/0.18
KLH + 100 ug	2	10.90/9.44	88.05/1.47	66.29/0.07	11.39/9.63	8.47/0.15	4.85/0
GPI-100	3	10.59/10.81	93.34/0.93	83.12/0.09	10.42/9.85	7.42/1.16	3.15/0.02
(100 ul/mice)	4	10.47/9.70	94.57/21.92	85.52/0.47	9.70/11.11	10.99/7.79	3.47/0.71
	5	12.29/10.03	99.77/6.12	99.79/0.77	*11.05/10.49	63.71/1.82	28.90/0.71
5. 3 ug STn(c)-KLH +	1	9.56/9.77	13.95/1.74	1.29/0.13	10.10/10.73	95.14/4.42	86.94/0.50
100 ug GPI-100	2	9.91/10.59	15.95/1.79	12.70/0.23	10.20/11.18	98.65/19.42	88.58/0.59
(100 ul/mice)	3	10.72/10.55	6.88/2.81	6.23/0.02	11.31/11.46	97.78/31.98	95.31/0.36
	4	9.62/9.52	4.62/1.69	3.18/0.14	10.11/9.78	94.14/1.19	86.65/0.09
	5	10.77/10.71	17.96/2.16	7.17/0.3	10.71/0.05	97.66/7.28	87.09/0.38
6. 3 ug Tf(c)-KLH +	1	10.84/10.39	13.89/5.73	5.86/0.23	9.64/10.31	7.66/4.74	3.85/0.24
100 ug GPI-100	2	9.22/11.34	31.03/4.44	31.75/0.10	9.65/10.15	9.01/1.17	3.98/0.18
(100 ul/mice)	3	10.59/10.11	4.05/1.05	0.73/0.02	10.12/9.92	4.97/2.77	0.90/0.04
	4	10.26/10.63	6.72/7.10	2.19/0.19	9.72/10.79	3.85/3.58	2.25/0.16
	5	12.0/10.90	9.11/6.28	5.18/0.18	*11.54/10.09	11.71/5.0	12.76/0.54

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7. 3 ug Tn(c)-KLH +	1	9.60/10.44	3.64/8.41	0.62/0.37	10.65/10.46	9.91/5.07	3.02/0.32
100 ug GPI-100	2	10.54/10.46	12.94/3.24	20.35/0.17	11.50/10.85	4.81/0.48	5.33/0.05
(100 ul/mice)	3	10.51/10.46	4.72/23.22	0.16/1.22	10.70/9.68	2.37/1.33	0.04/0.02
	4	10.97/9.48	8.50/17.77	4.52/0.16	10.94/10.97	18.50/1.81	4.18/0.12
	5	10.79/10.72	46.27/32.77	9.46/2.04	11.40/10.16	25.42/16.84	16.5/22.80
*DU145		+control	IE-3	91.67, 95.15	+control	IE-3	95.73%
			MLS128	77.07%		HMFG1	38.34%
			MLS132	19.92, 4.52		49H8	84.90%
8. Heptavalent-KLH +	1	10.84/11.21	89.44/17.63	69.48/0.47	10.29/10.63	97.79/49.78	90.80/0.46
100 ug GPI-100	2	9.55/9.54	96.98/25.87	83.67/1.12	9.97/10.58	95.44/74.27	83.11/0.35
(200 ul/mice)	3	9.94/10.88	96.53/32.44	88.36/0.79	10.56/10.91	95.39/40.29	85.79/0.49
	4	10.90/10.72	99.47/5.72	96.61/0.45	10.69/10.42	97.65/16.44	88.77/0.28
	5	10.37/10.17	75.64/10.67	63.57/0.11	10.58/10.14	93.93/15.49	71.06/1.36
9. Heptavalent-KLH +	1	10.65/10.57	74.25/12.81	69.56/1.07	10.93/10.21	99.45/8.83	85.59/0.38
100 ug GPI-100	2	10.20/10.97	98.22/16.51	81.61/0.91	10.86/10.78	96.70/22.94	66.20/0.50
(batch J)	3	9.96/9.68	89.36/1.03	60.88/0.22	9.79/9.80	95.10/0.43	53.45/0.03
(200 ul/mice)	4	10.77/10.75	82.25/8.95	27.80/0.09	10.58/10.28	92.21/1.17	65.13/0.18
	5	10.95/10.77	77.49/10.19	62.39/0.13			
10. Heptavalent-KLH +	1	10.99/10.32	84.54/8.61	39.91/0.49	10.63/10.92	93.11/38.33	63.61/1.57
100 ug GPI-100 +	2	10.09/10.92	97.83/11.79	94.21/0.16	10.72/10.68	97.64/12.52	77.95/0.05
polysorbate 80	3	11.32/10.32	97.74/15.67	89.24/0.30	9.53/10.16	89.84/2.13	63.96/0.19
(200 ul/mice)	4	9.76/10.59	92.51/15.57	82.83/0.10	11.50/10.03	60.13/14.56	8.56/1.59
	5	10.33/10.05	80.12/11.34	70.98/0.30			
11. Heptavalent-KLH +	1	10.96/10.08	91.06/1.64	55.98/0.02	9.76/10.81	26.58/2.13	5.42/0.16
10 ug QS-21	2	9.88/10.41	94.84/2.32	72.07/0.02	9.80/11.58	88.16/22.37	34.36/0.94
(200 ul/mice)	3	11.0/10.57	99.34/9.99	93.96/0.84	9.86/10.79	87.20/11.70	76.90/0.14
	4	11.05/10.65	88.58/3.78	61.69/0.13	10.07/10.45	71.56/3.79	26.42/0.15
	5	10.86/10.27	81.44/6.27	69.68/0.14			
12. Heptavalent-KLH +	1	18.54/11.29	67.73/1.59	26.33/0.18	10.98/9.97	16.98/1.97	6.40/0.09
3 ug ER803022	2	9.69/9.60	74.76/0.72	33.45/0.08	10.62/10.33	36.76/2.05	3.73/0.07
(200 ul/mice)	3	9.65/10.62	91.62/2.89	46.96/0.31	10.16/10.03	28.33/4.88	3.26/0.10
	4	10.78/10.0	22.58/4.14	9.51/0.36			
	5	10.90/10.94	48.15/7.29	10.64/0.23			
13. Heptavalent-KLH +	1	10.08/10.56	74.85/8.71	40.57/0.24	10.96/10.20	64.39/3.85	7.58/0.17
10 ug ER803732	2	11.03/10.91	36.35/5.07	4.79/0.07	9.84/10.19	3.67/11.73	0.47//0.73
(200 ul/mice)	3	10.22/10.54	65.94/30.22	7.87/0.63	10.83/10.07	66.06/25.19	8.66/0.82
	4	10.75/10.07	91.69/12.15	22.96/0.41			
	5	10.87/11.04	35.45/6.88	1.38/0.11			
14. 30 ug KLH +	1	9.42/11.35	13.72/9.84	10.08/2.29	10.85/10.49	5.49/4.48	3.29/2.53
100 ug GPI-100	2	12.14/11.31	17.50/2.09	6.41/0.0	10.58/10.44	10.57/3.53	14.38/0.21
(200 ul/mice)	3	9.82/10.97	1.64/1.05	0.13/0.02	9.19/11.45	15.10/1.03	11.63/0.01
	4	11.20/11.84	13.25/2.14	10.05/0.12			
	5	10.51/9.95	8.98/5.40	1.38/0.06			

Heptavalent: Tn(c) (3 ug), TF(c) (3 ug), sTn(c) (3 ug), MUC1-1G5 (3 ug), Ley (10 ug), Globo H (10 ug), and GM2 (l0 ug).
New GPI-100 used for all groups except group 9 (batch J).
Polysorbate 80, 4 mg/ml in final vaccine.

Third Series of Experiments

Polyvalent Conjugate Vaccine for Cancer

Tumor-specific antigens have been identified and pursued as targets for vaccines. The inventors' previous work has shown that monovalent vaccines utilizing the tumor antigens Globo H, Lewisy, GM2, glycosylated MUC-1, Tn(c), sTn(c), or TF(c) conjugated to KLH to be safe with local erythema and edema but minimal systemic toxicities. As a result of vaccination with these monovalent vaccines, most patients generated specific high titer IgM or IgG antibodies against the respective antigen-KLH conjugates. The present invention provides multivalent vaccines wherein the components of the monovalent vaccine are combined and administered with an adjuvant as treatment for cancer.

Vaccines consisting of a combination of tumor antigens administered with a saponin immunological adjuvant QS-21 or GPI-0100. The antigens are glycosylated MUC-1-G5, Globo H, GM2, Le^y, Tn(c), sTn(c), and TF(c). In each case the antigen is conjugated to Keyhole Limpet Hemocyanin (KLH).

The preferred ranges of the antigen and adjuvant doses are as follows:

Glycosylated MUC-1-G5: 0.1 to 30 g;
Globo H, 0.1 to 100 g;
GM2: 0.1 to 100 g;
Le^y: 0.1 to 60 g;
Tn(c): 0.1 to 100 g;
sTn(c): 0.1 to 100 g;
TF(c): 0.1 to 30 g;
QS-21: 25-200 g;
GPI-0100:1-25 mg.

Example 1

Comparison of the Immune Response after Immunization with Monovalent and Hexavalent-KLH Conjugate Vaccines Against Prostate Cancer

The immune response of the five initial patients receiving hexavalent vaccine with the immune responses of patients who had previously been immunized with the respective monovalent vaccines is compared in the following five tables. Shown are the reciprocal mean peak ELISA titer for IgM and IgG after immunization and FACS assay (% of positive cells/mean intensity) using the MCF-7 tumor cell line. The comparison for GM2-KLH is pending. Comparing the responses induced by monovalent and hexavalent vaccines, there was no significant difference in the antibody responses against any of the five antigens tested to date. Combination of six individual conjugates into a single vaccine does not significantly change the antibody response against the individual antigens.

Hexavalent Versus Monovalent: Prostate
Aug. 6, 2001

Trial	Patient	Patient Sera	Elisa (TF-HSA) IgM	Elisa (TF-HSA) IgG	MCF-7 FACS IgM %/Mean
protocol 98-048	M1	week 1	10	10	10/37
TF(c)-KLH + QS21		week 7	1280+	160	11/39
dosage: 1 μg		week 9	1280	40	17/50
	M2	week 1	10	0	10/21
		week 7	1280++	10	52/54
		week 9	1280+++	10	72/64
	M3	week 1	0	0	11/135
		week 7	1280++	160	2/28
		week 9	1280++	1280	6/104
	M4	week 1	0	0	10/32
		week 7	160	10	18/43
		week 9	160	160	26/47
	M5	week 1	0	0	10/36
		week 7	320	0	9/28
		week 9	320	0	8/22
protocol 00-064	H1	week 1	20	0	11/64
Hexavalent Conjgate + QS21		week 7	1280	160	7/55
TF(c)dosage: 3 μg		week 12	1280	160	3/31
	H2	week 1	40	10	9/37
		week 7	1280	160	21/50
		week 12	640	160	17/43
	H3	week 1	40	0	10/35
		week 7	1280	20	54/65
		week 12	640	40	34/47
	H4	week 1	80	0	10/26
		week 7	1280	80	22/43
		week 12	1280	20	19/54
	H5	week 1	0	0	10/13
		week 7	1280	320	57/26
		week 12	1280	320	47/22
Controls	C1	Aug. 27, 1999	1280	1280	

Hexavalent Versus Monovalent: Prostate
Aug. 10, 2001

Trial	Patient	Patient Sera	Elisa (Tn-HSA) IgM	Elisa (Tn-HSA) IgG	MCF-7 FACS IgM %/Mean
protocol 98-002	M1	week 1	0	80	10/22
Tn(c)-KLH + QS21		week 7	40	2560	7/18
dosage: 3 μg		week 9	80	2560	9/21
	M2	week 1	10	80	11/26
		week 7	320	5120	32/40
		week 9	640	5120	37/42
	M3	week 1	20	40	11/25
		week 7	640	640	12/26
		week 9	1280	1280	13/27
	M4	week 1	40	40	10/49
		week 7	640	1280	9/40
		week 9	1280	1280	8/39
	M5	week 1	10	80	11/25
		week 7	1280	5120	12/26
		week 9	1280	5120	13/27
protocol 00-064	H1	week 1	80	0	11/64
Hexavalent Conjgate + QS21		week 7	320	1280	7/55
Tn dosage: 3 μg		week 12	320	1280	3/31
	H2	week 1	320	10	9/37
		week 7	1280	320	21/50
		week 12	1280	320	17/43
	H3	week 1	10	0	10/35
		week 7	320	640	54/65
		week 12	640	640	34/47
	H4	week 1	40	160	10/26
		week 7	640	5120	22/43
		week 12	320	5120	19/54

-continued

Hexavalent Versus Monovalent:
Prostate
Aug. 10, 2001

Trial	Patient	Patient Sera	Elisa (Tn-HSA) IgM	IgG	MCF-7 FACS IgM %/Mean
	H5	week 1	0	20	10/13
		week 7	320	640	57/26
		week 12	320	640	47/22
Controls	C2		1280+	640	

Hexavalent Versus Monovalent:
Prostate
Aug. 17, 2001

Trial	Patient	Patient Sera	Elisa (GloboH) IgM	IgG	MCF-7 FACS IgM %/Mean
PROTOCOL 96-055	M1	week 1	0	0	11/40
GloboH-KLH + QS21		week 7	20	0	9/30
dosage: 10 μg		week 9	20	0	9/40
	M2	week 1	0	0	11/33
		week 7	0	0	12/42
		week 9	0	0	14/52
	M3	week 1	20	0	
		week 7	640	0	
		week 9	640	0	
	M4	week 1	10	0	11/26
		week 7	160	0	19/39
		week 9	160	0	25/39
	M5	week 1	40	0	10/26
		week 7	160	0	17/38
		week 9	off study	off study	
PROTOCOL 00-064	H1	week 1	40	0	11/41
Hexavalent Conjgate + QS21		week 7	160	0	9/36
GloboH dosage: 10 μg		week 12	160	80	6/27
	H2	week 1	160	0	11/33
		week 7	640	0	11/36
		week 12	320	0	15/40
	H3	week 1	40	0	11/29
		week 7	20	0	56/59
		week 12	20	0	43/46
	H4	week 1	80	0	10/34
		week 7	160	0	21/46
		week 12	80	0	16/44
	H5	week 1	40	0	10/15
		week 7	80	0	64/35
		week 12	80	0	45/27
Controls	C3	week 26		640	
	C4		1280		

Hexavalent Versus Monovalent:
Prostate
Aug. 31, 2001

Trial	Patient	Patient Sera	Elisa Ley-Cer IgM	IgG	MCF-7 FACS IgM %/Mean
PROTOCOL 00-075	M1	week 1	0	0	11/23
LewY-MMCCH-KLH		week 7	0	0	13/26
dosage: 20 μg		week 9	0	0	24/92
	M2	week 1	0	0	10/25
		week 7	0	0	20/32
		week 9	0	0	14/24
	M3	week 1	0	0	11/30
		week 7	40	80	8/20
		week 9	20	40	14/41
	M4	week 1	0	0	11/46
		week 7	0	0	10/50
		week 9	0	10	9/44

-continued

Hexavalent Versus Monovalent:
Prostate
Aug. 31, 2001

Trial	Patient	Patient Sera	Elisa Ley-Cer IgM	IgG	MCF-7 FACS IgM %/Mean
	M5	week 1	0	0	10/50
		week 7	40	0	3/30
		week 9	0	10	18/63
PROTOCOL 00-064	H1	week 1	0	0	11/41
Hexavalent		week 7	0	0	9/36
Conjgate + QS21		week 12	0	0	6/27
Ley dosage: 10 μg	H2	week 1	0	0	11/33
		week 7	0	0	11/36
		week 12	0	0	15/40
	H3	week 1	0	0	11/29
		week 7	10	0	56/59
		week 12	0	0	43/46

-continued

Hexavalent Versus Monovalent:
Prostate
Aug. 31, 2001

Trial	Patient	Patient Sera	Elisa Ley-Cer IgM	IgG	MCF-7 FACS IgM %/Mean
	H4	week 1	0	10	10/34
		week 7	10	10	21/46
		week 12	10	0	16/44
	H5	week 1	0	0	10/15
		week 7	0	0	64/35
		week 12	0	0	45/27
Controls	C5		2560		
	C6			640	

Hexavalent Versus Monovalent:
Prostate
Aug. 19, 2001

Trial	Patient	Patient Sera	Elisa (MUC33G5) IgM	IgG	MCF-7 FACS IgM %/Mean
PROTOCOL 99-23	M1	week 1	0	0	10/25
MUC33G 5 site - KLH + QS21		week 7	160	640	17/31
dosage: 3 μg		week 9	160	640	31/45
	M2	week 1	0	0	10/29
		week 7	2560	640	38/53
		week 9	2560	640	35/43
	M3	week 1	0	20	10/28
		week 7	2560	160	36/57
		week 9	2560	320	43/60
	M4	week 1	0	0	11/41
		week 7	2560	80	12/35
		week 9	640	320	12/38
	M5	week 1	0	0	11/30
		week 7	40	0	61/65
		week 9	40	80	59/67
PROTOCOL 00-064	H1	week 1	0	0	11/41
Hexavalent Conjgate + QS21		week 7	20	640	9/36
MUC33 dosage: 3 μg		week 12	0	160	6/27
	H2	week 1	0	0	11/33
		week 7	0	40	11/36
		week 12	0	80	15/40
	H3	week 1	0	0	11/29
		week 7	160	160	56/59
		week 12	40	320	43/46
	H4	week 1	0	20	10/34
		week 7	40	320	21/46
		week 12	40	160	16/44
	H5	week 1	0	0	10/15
		week 7	40	160	64/35
		week 12	0	80	45/27
Controls	C7		2560	640	

Immunization of mice with Heptavalent-KLH Conjugates* plus GPI-100 or QS-21
Nov. 30, 2001
Mean Value:

		ELISA				FACS MCF-7						LSC #LSC								
		Pre		Post		Pre		Post		1:200		Pre		Post		1:200		CDC(MCF-7)		
Group #	Antigen	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	Mice #	Pre	Post
1. 3 ug GM2-KLH + 100 ug GPI-100 (100 ul/ mice)	Tn Tf sTn Muc1-G5 Ley globo H GM2	0	0	0	0	10.43%	10.44%	9.40%	8.97%	6.47%	0.55%	10.59%	10.60%	6.41%	1.92%	3.63%	0.22%			
		Pre		Post		Pre		Post		1:200		Pre		Post		1:200				
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	Mice #	Pre	Post
2. 3 ug GloboH-KLH + 100 ug GPI100 (100 ul/ mice)	Tn Tf sTn Muc1-G5 Ley globo H GM2	0	0	640	5120	10.77% 10.08% (mice# 1 retested.)	10.57% 10.57%	15.22% 8.01%	22.50% 32.59%	13.47% 6.86%	2.78% 7.39%	10.33%	10.75%	7.08%	2.46%	4.70%	0.25%			
		Pre		Post		Pre		Post		1:200		Pre		Post		1:200				
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	Mice #	Pre	Post
3. 3 ug Ley-KLH + 100 ug GPI-100 (100 ul/ mice)	Tn Tf sTn Muc1-G5 Ley globo H GM2	0	0	640	2560	11.15%	10.34%	67.27%	34.02%	20.32%	1.43%	10.79%	9.63%	74.13%	40.76%	11.12%	2.26%	3-1 3-2 3-3 3-4 3-5	5.94% 4.47% 4.49% 22.5% 11.02%	46.42% 75.05% 9.21% 119.0% 61.7%

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		Pre		Post		Pre		Post		1:200		Pre		Post		1:200				
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	Mice #	Pre	Post
4. 3 ug Muc-1G-5-KLH + 100 ug GPI-100 (100 ul/ mice)	Tn Tf sTn Muc1-G5 Ley globo H GM2	0	0	2560+	1280	11.12%	10.12%	94.77%	7.99%	86.54%	0.46%	10.25%	10.12%	7.93%	2.55%	3.44%	0.23%	4-1 4-2 4-3 4-4 4-5	3.30% 6.85% 14.0% 4.49% 4.23%	

		Pre		Post		Pre		Post		1:200		Pre		Post		1:200				
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	Mice #	Pre	Post
5. 3 ug STn(c)-KLH + 100 ug GPI-100 (100 ul/ mice)	Tn Tf sTn Muc1-G5 Ley globo H GM2	0	0	5120	320	10.12%	10.23%	11.88%	2.04%	6.11%	0.16%	10.49%	8.64%	96.67%	12.86%	88.91%	0.38%	5-1 5-2 5-3 5-4 5-5	6.83% 10.91% 6.36% 7.11% 4.37%	

		Pre		Post		Pre		Post		1:200		Pre		Post		1:200				
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	Mice #	Pre	Post
6. 3 ug Tf(c)-KLH + 100 ug GPI-100 (100 ug/ mice)	Tn Tf sTn Muc1-G5 Ley globo H GM2	0	0	5120	320	10.58%	10.67%	10.56%	4.92%	9.14%	0.14%	9.78%	10.29%	6.37%	3.06%	2.74%	0.16%			

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		Pre		Post		Pre		Post		1:200		Pre		Post		1:200				
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	Mice #	Pre	Post
7. 3 ug Tn(c)-KHL + 100 ug GPI-100 (100 ug/ mice)	Tn Tf sTn Muc1-G5 Ley globo H GM2 KLH	0	0	5120+	2560	10.48%	10.31%	15.21%	17.10%	7.02%	0.79%	11.04%	10.42%	12.20%	5.11%	5.81%	4.66%			

		Pre		Post		Pre		Post		1:200		Pre		Post		1:200				
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	Mice #	Pre	Post
8. Hepta-valent-KLH + 100 ug GPI100 (200 ul/ mice)	Tn	0	10	5120+	320	10.32%	10.5%	91.61%	18.46%	80.34%	0.59%	10.43%	10.54%	96.04%	39.25%	83.91%	0.59%	8-1	0.75%	2.99%
	Tf	0	10	5120	320	10.63%	10.14%	98.92%	24.41%	85.27%	7.44%							8-2	1.0%	12.63%
	sTn	0	0	2560	640	(mice #1 re-tested)												8-3	−2.23%	44.48%
	Muc1-G5	0	0	5120++	80													8-4	−3.12%	24.77%
	Ley	0	0	80	160													8-5	7.65%	27.28%
	globo H	0	80	10	1280													VK9		2.05%
	GM2	0	0	0	10													3S193		82.2%
																		anti-GM2		71.2%
																		696		32.2%

		Pre		Post		Pre		Post		1:200		Pre		Post		1:200				
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	Mice #	Pre	Post
9. Hepta-valent-KLH + 100 ug GPI (old batch) (200 ul/ mice)	Tn	0	0	2560	40	10.51%	10.55%	84.31%	9.89%	60.45%	0.48%	10.40%	10.21%	92.22%	16.68%	63.24%	0.25%	9-1	−2.79%	34.02%
	Tf	0	0	5120	40													9-2	2.96%	9.42%
	sTn	0	0	320	160													9-3	4.50%	11.41%
	Muc1-G5	0	0	1280	0													9-4	6.74%	9.51%
	Ley	0	0	40	320													9-5	4.01%	39.91%
	globo H	0	80	0	320													3S193		97.83%
																		B72.3		5.34%
	GM2	0	0	0	0													HMFG.1		3.01%

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		Pre		Post		Pre		Post		1:200		Pre		Post		1:200				
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	Mice #	Pre	Post
10. Hepta-valent-KHL + 100 ug GPI-100 + poly-sorbate 80 (200 ul/mice)	Tn	0	0	5120+	640	10.50%	10.44%	90.55%	12.59%	75.43%	0.27%	10.67%	10.24%	85.22%	11.17%	54.16%	0.74%	10-1	−6.29%	−3.3%
	Tf	0	0	5120	320															
	sTn	0	0	640	640															
	Muc1-G5	0	0	5120	80															
	Ley	0	0	0	320															
	globo H	0	160	20	1280															
	GM2	0	0	0	0															

		Pre		Post		Pre		Post		1:200		Pre		Post		1:200				
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	Mice #	Pre	Post
11. Hepta-valent-KHL + 10 ug QS-21 (200 ul/mice)	Tn	0	20	5120+	160	10.75%	10.39%	90.96%	4.8%	70.67%	0.23%	9.92%	10.74%	70.28%	8.33%	40.71%	0.29%	11-1	1.84%	10.93%
	Tf	0	80	5120+	640													11-2	0.71%	3.20%
	sTn	0	0	2560	320													11-3	1.31%	5.88%
	Muc1-G5	0	0	2560	160													11-4	−1.58%	18.36%
	Ley	0	0	10	10													11-5	10.28%	27.09%
	globo H	0	80	40	640															
	GM2	0	0	0	0															

		Pre		Post		Pre		Post		1:200		Pre		Post		1:200				
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	Mice #	Pre	Post
12. Hepta-valent-KLH + 3 ug ER803022 (200 ul/mice)	Tn	0	0	1280	160	11.89%	10.49%	60.97%	3.33%	25.38%	0.23%	10.42%	10.29%	29.79%	4.55%	5.65%	0.14%			
	Tf	0	0	1280	80															
	sTn	0	0	80	80															
	Muc1-G5	0	0	80	20															
	Ley	0	0	0	20															
	globo H	20	160	80	640															
	GM2	0	0	0	0															

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		Pre		Post		Pre		Post		1:200		Pre		Post		1:200				
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	Mice #	Pre	Post
13. Hepta-valent-KLH + 10 ug ER803732 (200 ul/ mice)	Tn	0	20	160	160	10.59%	10.62%	60.86%	12.60%	15.51%	0.29%	10.50%	10.24%	45.30%	11.86%	5.60%	0.60%			
	Tf	0	10	320	160															
	sTn	0	40	20	160															
	Muc1-G5	0	0	160	80															
	Ley	0	0	0	80															
	globo H	20	80	80	320															
	GM2	0	0	0	0															

		Pre		Post		Pre		Post		1:200		Pre		Post		1:200				
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	Mice #	Pre	Post
14. 30 ug KLH + 100 ug GPI-100 (100 ul/ mice)	Tn	0	0	0	160	10.62%	11.08%	11.02%	4.10%	5.61%	0.45%	10.32%	10.68%	9.09%	2.47%	7.96%	0.73%			
	Tf	0	40	0	320															
	sTn	0	0	0	80															
	Muc1-G5	0	10	0	160															
	Ley	0	0	0	0															
	globo H	0	160	10	640															
	GM2	0	0	0	160															
	KLH	0	10	5120++	320															

Immunization of mice with Heptavalent-KLH Conjugates* plus QS-21 or GPI 100 with or without mAb 9H10 against CTLA-4.
Mean Value:

		ELISA (mean)				ELISA (median)				FACS MCF-7						⟹	AVERAGE	
		Pre		Post		Pre		Post		Pre		Post		1:200		⟹	MEDIAN	
Group #	Antigen	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM		+CONTROL	
1. Hepta-valent-KLH + 10 ug QS21	Tn	0	0	57440	640	0	0	25600	640	9.89%	9.78%	95.87%	77.77%	78.03%	16.09%		VK-9	40.08%
	Tf	0	0	10240++	260	0	0	10240+	160	9.39%	9.94%	96.40%	80.96%	79.70%	8.16%		MLS128	97.56%
	sTn	0	0	3520	580	0	0	1280	320								antiGM2	####
	Muc1-G5	0	0	1280	80	0	0	1280	80								MBr-1	####
	Ley	0	32	1920	2560	0	0	1280	1280									
	globo H	0	48	672	128	0	80	640	160									
	GM2																	

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		Pre		Post		Pre		Post		Pre		Post		1:200	
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
2. Hepta-valent-KLH + 100 ug GPI100	Tn	0	0	19200	840	0	0	25600	320	10.15%	10.36%	92.97%	79.87%	66.63%	28.35%
	Tf	0	0	10240+	263	0	0	10240+	160	10.10%	10.35%	95.99%	93.53%	69.88%	26.96%
	sTn	0	0	720	360	0	0	320	80						
	Muc1-G5	0	0	1520	80	0	0	2560	160						
	Ley	0	0	3920	680	0	0	5120	640						
	globo H	0	0	320	176	0	0	80	160						
	GM2														

		Pre		Post		Pre		Post		Pre		Post		1:200	
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
3. Hepta-valent-KLH + 100 ug GPI-100 (Lyo-phylized)	Tn	0	0	32000	480	0	0	25600	320	10.29%	10.14%	92.85%	62.94%	65.36%	18.34%
	Tf	0	0	10240+	340	0	0	10240+	320	10.20%	10.23%	92.35%	58.56%	63.59%	14.46%
	sTn	0	0	1200	260	0	0	1280	320						
	Muc1-G5	0	0	1120	0	0	0	320	0						
	Ley	0	0	320	720	0	0	480	640						
	globo H	0	0	128	440	0	0	0	200						
	GM2														

		Pre		Post		Pre		Post		Pre		Post		1:200	
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
4. Hepta-valent-KLH + 100 ug GPI100' + poly-sorbate 80	Tn	0	0	38400	400	0	0	25600	160	10.14%	10.18%	91.34%	57.09%	70.79%	6.70%
	Tf	0	0	10240+	140	0	0	10240+	160	10.22%	10.29%	90.80%	61.70%	67.27%	4.98%
	sTn	0	0	1960	200	0	0	1280	320						
	Muc1-G5	0	0	4000	20	0	0	2560	0						
	Ley	0	0	1440	960	0	0	1280	640						
	globo H	0	40	100	224	0	40	120	160						
	GM2														

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		Pre		Post		Pre		Post		Pre		Post		1:200	
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
5. Hepta-valent-KLH + 100 ug GPI100 Cytoxan 25 mg/Kg (I.P.) Day −1	Tn	0	0	61400	808	0	0	25600	640	9.92%	10.30%	89.55%	72.17%	64.38%	12.43%
	Tf	0	0	10240++	560	0	0	10240++	640	10.19%	10.19%	90.87%	70.90%	66.42%	14.32%
	sTn	0	0	520	480	0	0	640	320						
	Muc1-G5	0	0	3840	0	0	0	2560	0						
	Ley	0	0	480	200	0	0	0	80						
	globo H	0	0	32	540	0	0	0	400						

		Pre		Post		Pre		Post		Pre		Post		1:200	
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
6. Hepta-valent-KLH + 100 ug GPI100 mAb CTLA-4 in vaccine (100 ug/mice) Day 0 Day 7 & 14 no CTLA-4	Tn	0	0	32000	250	0	0	25600	160	10.33%	10.08%	90.59%	65.89%	62.40%	17.62%
	Tf	0	0	10240+	1040	0	0	10240+	1280	10.26%	10.01%	91.50%	62.97%	63.04%	8.00%
	sTn	0	0	6240	180	0	0	1280	200						
	Muc1-G5	0	0	1950	40	0	0	2560	40						
	Ley	0	0	180	2600	0	0	40	40						
	globo H	0	0	0	240	0	0	0	240						
	GM2														

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		Pre		Post		Pre		Post		Pre		Post		1:200	
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
7. Hepta-valent-KLH + 100 ug GPI100 mAb CTLA-4 in vaccine (100 ug/ mice) Day 0 & 7 Day 14 no CTLA-4	Tn	0	0	32000	1600	0	0	25600	1280	10.05%	10.31%	90.31%	85.42%	67.29%	29.21%
	Tf	0	0	10240+	1920	0	0	10240+	1280	9.64%	10.41%	90.01%	85.58%	69.08%	21.26%
	sTn	0	0	2880	100	0	0	2560	80						
	Muc1-G5	0	0	6400	60	0	0	5120	80						
	Ley	0	0	32	208	0	0	0	160						
	globo H	0	0	32	220	0	0	0	240						
	GM2														

		Pre		Post		Pre		Post		Pre		Post		1:200	
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
8. Hepta-valent-KLH + 100 ug GPI100 mAb CTLA-4 not in vaccine I.P. day −1, 0, 1	Tn	0	0	85333	40	0	0	51200+	40	9.69%	10.15%	91.29%	45.33%	63.00%	6.19%
	Tf	0	0	10240++	370	0	0	10240++	320	9.63%	10.09%	90.20%	45.21%	50.05%	7.00%
	sTn	0	0	480	0	0	0	640	0						
	Muc1-G5	0	0	3413	0	0	0	2560	0						
	Ley	0	0	320	106	0	0	160	0						
	globo H	0	0	53	240	0	0	0	80						
	GM2														

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		Pre		Post		Pre		Post		Pre		Post		1:200	
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
9. Hepta-valent-KLH + 100 ug GPI100 Control mAb ROR-g2 100 ug/ mice I.P. day −1, 0, 1	Tn	0	0	56320	640	0	0	51200	640	10.25%	10.13%	93.16%	90.64%	73.15%	45.88%
	Tf	0	0	10240+++	2720	0	0	10240+++	2560	10.27%	10.21%	94.17%	95.54%	76.62%	30.38%
	sTn	0	0	3040	5200	0	0	1280	320						
	Muc1-G5	0	0	10240	160	0	0	10240	160						
	Ley	0	0	144	464	0	0	80	160						
	globo H	0	0	112	848	0	0	80	640						
	GM2														

		Pre		Post		Pre		Post		Pre		Post		1:200	
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
10. Hepta-valent-KLH + 100 ug GPI100 100 ug/ mice	Tn	0	0	81920	320	0	0	51200	160	10.42%	9.98%	88.03%	76.96%	67.02%	33.26%
	Tf	0	0	10240++	920	0	0	10240++	320	10.50%	10.01%	87.97%	90.19%	64.58%	24.15%
	sTn	0	0	2240	1440	0	0	2560	160						
	Muc1-G5	0	0	4160	40	0	0	2560	0						
	Ley	0	144	144	2640	0	160	80	320						
	globo H	0	0	0	224	0	0	0	160						
	GM2														

Example 2

A Preclinical Study Comparing Approaches for Augmenting the Immunogenicity of a Heptavalent KLH-Conjugate Vaccine Against Epithelial Cancers

Previously using a series of monovalent vaccines, we have demonstrated that the optimal method for inducing an antibody response against cancer cell-surface antigens is covalent conjugation of the antigens to keyhole limpet hemocyanin (KLH) and the use of a saponin adjuvant. In preparation for testing a polyvalent (heptavalent)-KLH conjugate vaccine in the clinic, we have tested the impact on antibody induction against the 7 antigens of several variables described by others to augment immunogenicity. We explore here the impact of approaches for decreasing suppression of the immune response (low dose cyclophosphamide and anti-CTLA4 mAb), different saponin adjuvants (QS-21 and GPI-0100), and different methods of formulation (lyophilization and use of polysorbate 80). After two sets of experiments, these results are clear:

1) Immunization with the heptavalent-KLH conjugate vaccine induces high titers of antibodies against Tn (median ELISA titer IgM/IgG 320/10,240), sTn (640/2560), TF (320/5120), MUC1 (80/20,480) and globo H (1280/10), lower titers of antibodies against Lewis Y (160/80) and only occasional antibodies against GM2.

2) These antibodies reacted with the purified synthetic antigens by ELISA, and with naturally expressed antigens on the cancer cell surface by FACS.

3) Neither decreasing suppression with low dose cyclophosphamide or anti-CTLA4 mAb, nor changing the standard formulation by lyophilization or use of polysorbate 80 had any impact on antibody titers.

4) The two saponin adjuvants were comparably potent at our standard doses (QS-21 10 ug and GPI-0100 100 ug) but a third experiment comparing higher doses is in progress.

The high titers of antibodies against this heptavalent vaccine and the inability of these additional approaches to further augment antibody titers confirms that the combination of conjugation to KLH and use of a saponin adjuvant is sufficiently optimized for testing in the clinic.

There is a broad and expanding body of pre-clinical and clinical studies demonstrating that naturally acquired, actively induced, and passively administered antibodies are able to eliminate circulating tumor cells and micro metastases (1). Induction of antibodies against tumor antigens is more difficult than induction of antibodies against viral and bacterial antigens because most tumor antigens are normal or slightly modified auto antigens and because actively growing tumors may set in motion mechanisms which suppress the anti-cancer cell immune response. Consequently it may be necessary to overcome not only some level of tolerance but also some additional level of active suppression, making the immunization approach critical. We have previously reported that the optimal approach for induction of antibodies against gangliosides and a variety of other carbohydrate and peptide antigens is covalent attachment of the tumor antigen to an immunogenic carrier molecule (keyhole limpet hemocyanin (KLH) was optimal (2,3)) plus the use of a potent immunological adjuvant. In our previous experience saponin adjuvants such as QS-21 and GPI-0100 were the optimal adjuvants (4,5).

In preparation for clinical trials with a heptavalent KLH-conjugate vaccine we test here the impact of several variables including 1) vaccine formulation (lyophilization or the use of polysorbate 80), 2) decreasing suppression (low dose cyclophosphamide or anti-CTLA4 mAb), or 3) various doses of the two saponin adjuvants QS-21 and GPI-0100, on antibody titers against the individual antigens and tumor cells expressing these antigens.

Pathogen-free female BALE/c or C57BL/6 mice 6-10 weeks of age were obtained from the Jackson Laboratory (Bar Harbor, Me.). QS-21 was obtained from Aquila Biopharmaceuticals (Framingham, Mass. (now Antigenics Inc., NYC, NY)), GPI-0100 was obtained from Galenica Pharmaceuticals, Inc. (Birmingham, Ala.). Cytoxan (25 mg/kg) was purchased and injected IP one day prior to the first immunization. The hybridoma for murine monoclonal antibody CTLA-4 was obtained from Jim Allison (Berkeley, Calif.) and the mAb was prepared by Dr. Polly Gregor (MSKCC). The reactivity of mAb with CTLA-4 was confirmed. Polysorbate 80 was purchased.

Immunization of Mice: groups of five mice were immunized 3 times at one week intervals with the heptavalent vaccine containing 3 mcg of each of the 7 antigens covalently conjugated to KLH and mixed with GPI-0100 or QS-21 as indicated. Vaccines were administered subcutaneously over the lower abdomen. A 4th, booster, immunization was given at week 8.

Serological Assays: For the ELISA assay, glycosylated MUC1, globo H, Lewis Y or GM2, or Tn, sTn or TF conjugated to BSA, were coated on ELISA plates at an antigen dose of 0.1-0.2 mcg per well.

Phosphatase-conjugated goat anti-mouse IgG or IgM was added at a dilution of 1:200 (Southern Biotechnology Associates, Inc., Birmingham, Ala.). Antibody titer was the highest dilution yielding absorbance of 0.10 or greater.

F ACS analysis: MCF-7 human breast cancer cells expressing all seven antigens but especially Lewis Y and MUC1 and sTn, and LSC expressing especially Lewis Y, sTn and Tn, were used. Single cell suspensions of 5×10^7 cells/tube were washed in PBS with 3% fetal calf serum and incubated with 20 mcl of full strength or 1/200 diluted antisera for 30 minutes on ice. 20 microliters of 1/15 goat anti-mouse IgG or IgM labeled with FITC were all added and percent positive cells and mean fluorescent intensity (MFI) of stained cells analyzed using a F ACScan (Becton Dickenson, Calif.). Pre and post vaccination sera were analyzed together and the pretreatment percent positive cells set at 10%.

Comparision of the Immune Response after Immunization with Monovalent and Hexavalent-KLH Conjugate Vaccines Against Prostate Cancer

Glycolipid and glycoprotein differentiation antigens such as GM2, Globo H, Lewis y, Tn, TF, and mucin 1 (MUC1) are over-expressed on the cell surface of many tumors. Of the many approaches to immunization we have tested, covalent conjugation of antigens such as these to keyhole limpet hemocyanin (KLH) plus the use of immunological adjuvant QS-21 has been the optimal approach for inducing IgM and IgG antibodies. Immunization of patients with monovalent vaccines containing these antigens has demonstrated the consistent immunogenicity and safety of these vaccines. However, to overcome the heterogeneous nature of tumors, and of the immune response in different individuals, we have recently vaccinated a small group of patients (prostate cancer with rising PSA, but free of detectable disease) with a hexavalent-KLH vaccine containing GM2, Globo H, Le^y, Tn(c), TF(c) and glycosylated MUC1 individually conjugated with KLH and mixed with immunological adjuvant QS-21. The main objective of this presentation is to compare the immune response of the six initial patients receiving hexavalent vaccine with the immune responses of patients who had previously been immunized with the respective monovalent vaccines. All patients were vaccinated six times (weeks 1,2,3,7, 19 and 31) and bloods obtained pre treatment and on weeks 7 and 9 were tested at one time. RECIPRICOL MEAN PEAK ELISA TITER AFTER IMMUNIZATION IgM/IgG.

Antigen	GM2	Globo H	Le^y	Tn	TF	MUC1
Polyvalent vaccine	Pending	160/0	0/0	640/640	1280/160	40/320
Individual Vaccine	Pending	160/0	0/10	1280/2560	1280/160	2560/320

Because of the low response against Le^y, we are continuing studies aimed at creating a more immunogenic Le^y vaccine. Comparing the responses induced by monovalent and hexavalent vaccines, there was no significant difference in the antibody responses against any of the five antigens tested to date. Combination of six individual conjugates into a single vaccine does not significantly change the antibody response against the individual antigens.

Experiment 1: Median ELISA titers and FACS results
after vaccination of groups of 5 Balb/c mice with Heptzvalent-KLH conjugate

		ELISA(mean)				ELISA(median)				FACS MCF-7							
		Pre		Post		Pre		Post		Pre		Post		1:200			
Group	Anti-gen	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	+CONTROL	
1. Hepta-valent-KLH + 10 ug QS21	Tn	0	0	57440	640	0	0	25600	640	10%	10%	96%	78%	78%	16%	VK-9	40.08%
	Tf	0	0	10240++	260	0	0	10240+	160	9%	10%	96%	81%	80%	8%	MLS128	97.56%
	sTn	0	0	3520	580	0	0	1280	320							antiGM2	98.87%
	Muc1-G5	0	0	1280	80	0	0	1280	80							MBr-1	58.06%
	Ley	0	32	1920	2560	0	0	1280	1280								
	globo H	0	48	672	128	0	80	640	160								
	GM2																
2. Hepta-valent-KLH + 100 ug GPI100	Tn	0	0	19200	840	0	0	25600	320	10%	10%	93%	80%	67%	28%		
	Tf	0	0	10240+	260	0	0	10240+	160	10%	10%	96%	94%	70%	27%		
	sTn	0	0	720	360	0	0	320	80								
	Muc1-G5	0	0	1520	80	0	0	2560	160								
	Ley	0	0	3920	680	0	0	5120	640								
	globo H	0	0	320	176	0	0	80	160								
	GM2																
3. Hepta-valent-KLH + 100 ug GPI-100 (Lyo-phil-ized)	Tn	0	0	32000	480	0	0	25600	320	10%	10%	93%	63%	65%	18%		
	Tf	0	0	10240+	340	0	0	10240+	320	10%	10%	92%	59%	64%	14%		
	sTn	0	0	1200	260	0	0	1280	320								
	Muc1-G5	0	0	1120	0	0	0	320	0								
	Ley	0	0	320	720	0	0	480	640								
	globo H	0	0	128	440	0	0	0	200								
	GM2																
4. Hepta-valent-KLH + 100 ug GPI-100' + poly-sorbate 80	Tn	0	0	38400	400	0	0	25600	1601	10%	10%	91%	57%	71%	7%		
	Tf	0	0	10240+	140	0	0	10240+	60	10%	10%	91%	62%	67%	5%		
	sTn	0	0	1960	200	0	0	1280	320								
	Muc1-G5	0	0	4000	20	0	0	2560	0								
	Ley	0	0	1440	960	0	0	1280	640								
	globo H	0	40	100	224	0	40	120	160								
	GM2																
5. Hepta-valent-KLH + 100 ug GPI100 Cytoxan 25 mg/Kg (I.P.) Day −1	Tn	0	0	61400	808	0	0	25600	640	10%	10%	90%	72%	64%	12%		
	Tf	0	0	10240++	560	0	0	10240++	640	10%	10%	91%	71%	66%	14%		
	sTn	0	0	520	480	0	0	640	320								
	Muc1-G5	0	0	3840	0	0	0	2560	0								
	Ley	0	0	480	200	0	0	0	80								
	globo H	0	0	32	540	0	0	0	400								
	GM2																
6. Hepta-valent-KLH + 100 ug GPI100 mAb	Tn	0	0	32000	250	0	0	25600	160	10%	10%	91%	66%	62%	18%		
	Tf	0	0	10240+	1040	0	0	10240+	1280	10%	10%	92%	63%	63%	8%		
	sTn	0	0	6240	180	0	0	1280	200								
	Muc1-G5	0	0	1950	40	0	0	2560	40								
	Ley	0	0	180	2600	0	0	40	40								
	globo	0	0	0	240	0	0	0	240								

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Experiment 1: Median ELISA titers and FACS results
after vaccination of groups of 5 Balb/c mice with Heptzvalent-KLH conjugate

		ELISA(mean)				ELISA(median)				FACS MCF-7						
		Pre		Post		Pre		Post		Pre		Post		1:200		
Group	Anti-gen	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	+CONTROL
CTLA-4 in vaccine (100 ug/ mice) Day 0 Day 7 & 14 no CTLA-4	H															
7.	Tn	0	0	32000	1600	0	0	25600	1280	10%	10%	90%	85%	67%	29%	
Hepta-	Tf	0	0	10240+	1920	0	0	10240+	1280	10%	10%	90%	86%	69%	21%	
valent-	sTn	0	0	2880	100	0	0	2560	80							
KLH + 100 ug	Muc1-G5	0	0	6400	6	0	0	5120	80							
GPI100	Ley	0	0	32	208	0	0	0	160							
mAb CTLA-4 in vaccine (100 ug/ mice) Day 0 & 7 Day 14 no CTLA-4	globo H GM2	0	0	32	220	0	0	0	240							
8.	Tn	0	0	85333	40	0	0	51200+	40	10%	10%	91%	45%	63%	6%	
Hepta-	Tf	0	0	10240++	370	0	0	10240++	320	10%	10%	90%	45%	50%	7%	
valent-	sTn	0	0	480	0	0	0	640	0							
KLH + 100 ug	Muc1-G5	0	0	3413	0	0	0	2560	0							
GPI100	Ley	0	0	320	106	0	0	160	0							
mAb CTLA-4 not in vaccine I.P. day −1, 0, 1	globo H GM2	0	0	53	240	0	0	0	80							
9.	Tn	0	0	56320	640	0	0	51200	640	10%	10%	93%	91%	73%	46%	
Hepta-	Tf	0	0	10240+++	2720	0	0	10240+++	2560	10%	10%	94%	96%	77%	30%	
valent-	sTn	0	0	3040	5200	0	0	1280	320							
KLH + 100 ug	Muc1-G5	0	0	10240	160	0	0	10240	160							
GPI100	Ley	0	0	144	464	0	0	80	160							
Control mAb ROR-g2 100 ug/ mice I.P. day −1, 0, 1	globo H GM2	0	0	112	848	0	0	80	640							
10.	Tn	0	0	81920	320	0	0	51200	160	10%	10%	88%	77%	67%	33%	
Hepta-	Tf	0	0	10240++	920	0	0	10240++	320	11%	10%	88%	90%	65%	24%	
valent-	sTn	0	0	2240	1440	0	0	2560	160							
KLH + 100 ug	Muc1-G5	0	0	4160	40	0	0	2560	0							
GPI100	Ley	0	144	144	2640	0	160	80	320							
100 ug/ mice	globo H GM2	0	0	0	224	0	0	0	160							

Experiment 2: Median ELISA titers and FACS results against MCF-7 and LSC cells after vaccination groups of 5 C57BL/6 Mice with heptavalent-KLH conjugate vaccine

		ELISA				FACS with MCF-7						FACS with LSC					
												# LSC					
		Pre		Post		Pre		Post		1:200		Pre		Post		1:200	
Group #	Antigen	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
Heptavalent-KLH + 100 ug GPI100 (200 ul/mice)	Tn	0	10	5120+	320	10%	11%	91.61%	18.46%	80%	18%	10%	11%	96.04%	39.25%	84%	39%
	Tf	0	10	5120	320			99%	24%	82%	21%						
	sTn	0	0	2560	640												
	Muc1-G5	0	0	5120++	80												
	Ley	0	0	80	160												
	globo H	0	80	10	1280												
	GM2	0	0	0	10												
		Pre		Post		Pre		Post		1:200		Pre		Post		1:200	
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
Heptavalent-KLH + 100 ug GPI (old batch) (200 ul/mice)	Tn	0	0	2560	40	11%	11%	84.31%	9.89%	60%	10%	10%	10%	92.22%	16.68%	63%	17%
	Tf	0	0	5120	40												
	sTn	0	0	320	160												
	Muc1-G5	0	0	1280	0												
	Ley	0	0	40	320												
	globo H	0	80	0	320												
	GM2	0	0	0	0												
		Pre		Post		Pre		Post		1:200		Pre		Post		1:200	
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
Heptavalent-KLH + 100 ug GPI-100 + polysorbate 80 (200 ul/mice)	Tn	0	0	5120+	640	11%	10%	90.55%	12.59%	75%	13%	11%	10%	85.22%	11.17%	54%	11%
	Tf	0	0	5120	320												
	sTn	0	0	640	640												
	Muc1-G5	0	0	5120	80												
	Ley	0	0	0	320												
	globo H	0	160	20	1280												
	GM2	0	0	0	0												
		Pre		Post		Pre		Post		1:200		Pre		Post		1:200	
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
Heptavalent-KLH + 10 ug QS-21 (200 ul/mice)	Tn	0	20	5120+	160	11%	10%	90.96%	4.8%	71%	5%	10%	11%	70.28%	8.33%	41%	8%
	Tf	0	80	5120+	640												
	sTn	0	0	2560	320												
	Muc1-G5	0	0	2560	160												
	Ley	0	0	10	10												
	globo H	0	80	40	640												
	GM2	0	0	0	0												

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Experiment 2: Median ELISA titers and FACS results against MCF-7 and LSC cells after vaccination groups of 5 C57BL/6 Mice with heptavalent-KLH conjugate vaccine

		ELISA				FACS with MCF-7						FACS with LSC					
		Pre IgG	IgM	Post IgG	IgM	Pre IgG	IgM	Post IgG	IgM	1:200 IgG	IgM	Pre IgG	IgM	Post IgG	IgM	1:200 IgG	IgM
Heptavalent-KLH + 10 ug ER803732 (200 ul/mice)	Tn	0	20	160	160	11%	11%	60.86%	12.60%	16%	13%	11%	10%	45.30%	11.86%	6%	12%
	Tf	0	10	320	160												
	sTn	0	40	20	160												
	Muc1-G5	0	0	160	80												
	Ley	0	0	0	80												
	globo H	20	80	80	320												
	GM2	0	0	0	0												

Protocol 00-106:
Pilot Phase Trial: Vaccination of Patients Who Have Ovarian, Fallopian Tube or Peritoneal Cancer with A Polyvalent Vaccine-KLH Conjugate + QS-21

				Vaccine:													
				10 ug GM2		10 ug Globo-H		10 ug LeY		3 ug Muc1G5		3 ug Tn(c)		3 ug S-Tn(c)		3 ug TF(c)	
Patient Name Patient #	Vaccination	Serology	Sera #	Muc1-1G5 IgM	IgG	Globo-H IgM	IgG	LeY IgM	IgG	GM2 IgM	IgG	Tn(c) IgM	IgG	S-Tn(c) IgM	IgG	TF(c) IgM	IgG
				Jun. 12, 2001	Jun. 12, 2002	Jun. 27, 2002	Jun. 19, 2002	Jun. 21, 2002	Jun. 21, 2002	Jun. 24, 2002	Jun. 24, 2002					Jun. 25, 2002	Jun. 25, 2002
Patient 1	Jul. 3, 2001	Jul. 3, 2001	P7OVQ 2	0	0	0	0	0	0	0	0					0	20
	Jul. 10, 2001	Jul. 31, 2001	P7OVQ 6	2560	640	40	0	0	0	0	20					1280	160
	Jul. 17, 2001	Aug. 14, 2001	P7OVQ 9	2560	640	40	0	0	0	0	20					1280	160
	Aug. 14, 2001	Aug. 8, 2001	P7OVQ 15	2560	320	40	0	0	0	0	20					1280	320
		OFF TRIAL															
			(+) Control														
			M62	2560	640												
			Positve control				2560										
			S193(1 mg/ml)					6400									
			Positve control						2560								
			Positve control							640							
			Positve control								2560						
			Positve control													2560	2560
			Positve control			2560											
				Jun. 4, 2002	Jun. 4, 2002	Jun. 27, 2002	Jun. 26, 2002										
Patient 2	Jul. 3, 2001	Jul. 3, 2001	P7OVQ 3	0	0	0	0										
	Jul. 10, 2001	Jul. 31, 2001	P7OVQ 7	80	20	0	0										
	Jul. 17, 2001	Aug. 14, 2001	P7OVQ 10	40	20	0	0										
	Aug. 14, 2001	Aug. 28, 2001	P7OVQ 13	20	40	0	0										
		Sep. 25, 2001	P7OVQ 20	20	20	0	0										

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Experiment 2: Median ELISA titers and FACS results against MCF-7 and LSC cells after vaccination groups of 5 C57BL/6 Mice with heptavalent-KLH conjugate vaccine

				ELISA				FACS with MCF-7								FACS with LSC	
	Oct. 9, 2001	Oct. 9, 2001	P7OVQ 24	20	20	0	0										
		Oct. 23, 2001	P7OVQ 30	20	20	0	0										
		Jan. 11, 2002	P7OVQ 49	0	0	0	0										
		Jun. 7, 2002	P7OVQ 65			0	0										
			(+) Control														
			Positve control	2560	640												
			Positve control				2560										
			Positve control			640											
Patient Name Medical #	Vaccination	Serology	Sera #	Muc1-1G5 IgM	IgG	Globo-H IgM	IgG	LeY IgM	IgG	GM2 IgM	IgG	Tn(c) IgM	IgG	STn(c) IgM	IgG	Tf IgM	IgG
				Jun. 7, 2002	Jun. 7, 2002	Jun. 27, 2002	Jun. 26, 2002										
Patient 3	Jul. 10, 2001	Jul. 10, 2001	P7OVQ 4	0	0	20	0										
	Jul. 17, 2001	Aug. 7, 2001	P7OVQ 8	1280	160	160	0										
	Jul. 24, 2001	Aug. 21, 2001	P7OVQ 12	320	40	80	0										
	Aug. 21, 2001	Sep. 4, 2001	P7OVQ 18	160	80	80	0										
		Oct. 2, 2001	P7OVQ 22	80	40	40	0										
	Oct. 16, 2001	Oct. 16, 2001	P7OVQ 26	40	40	80	0										
		Oct. 30, 2001	P7OVQ 33	80	80	40	0										
		Jan. 8, 2002	P7OVQ 47	40	40	80	0										
			(+) Control														
			M62	2560	1280												
			Positve control				2560										
			Positve control			1280											
				Jun. 10, 2002	Jun. 10, 2002												
patient 4	Jul. 24, 2001	Jul. 24, 2001	P7OVQ 5	0	0												
	Jul. 31, 2001	Aug. 21, 2001	P7OVQ 14	1280	80												
	Aug. 7, 2001	Sep. 4, 2001	P7OVQ 17	1280	40												
	Sep. 4, 2001	Sep. 18, 2001	P7OVQ 23	160	20												
		Oct. 16, 2001	P7OVQ 27	160	20												
	Oct. 30, 2001	Oct. 30, 2001	P7OVQ 32	80	40												
		Nov. 13, 2001	P7OVQ 36	20	20												
		Jan. 22, 2002	P7OVQ 52	20	20												
		Mar. 26, 2002	P7OVQ 63	20	20												
			(+) Control														
			M62	1280	640												
				Jun. 12, 2002	Jun. 12, 2002	Jun. 27, 2002	Jun. 19, 2002	Jun. 21, 2002	Jun. 21, 2002	Jun. 24, 2002	Jun. 24, 2002					Jun. 25, 2002	Jun. 25, 2002
patient 5	Aug. 16, 2001	Aug. 16, 2001	P7OVQ 11	0	0	0	0	0	0	0	0					0	0
	Aug. 23, 2001	Sept. 27, 2001	P7OVQ 21	80	80	0	0	0	0	0	0					40	160

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Experiment 2: Median ELISA titers and FACS results against MCF-7 and LSC cells after vaccination groups of 5 C57BL/6 Mice with heptavalent-KLH conjugate vaccine

				ELISA		FACS with MCF-7						FACS with LSC	
	Aug. 30, 2001	Oct. 11, 2001	P7OVQ 25	40	80	0	0	0	0	0	0	0	160
	Sep. 27, 2001	OFF TRIAL											
			(+) Control										
			M62	2560	640								
			Positve control				2560						
			S193(1 mg/ml)					6400					
			Positve control						2560				
			Positve control							640			
			Positve control								2560		
			Positve control									2560	2560
			Positve control			2560							
				Jun. 17, 2002	Jun. 17, 2002								
Patient 6	Aug. 30, 2001	Aug. 30, 2001	P7OVQ 16	0	0								
	Sep. 6, 2001	Sep. 27, 2001	P7OVQ 19	160	20								
	Sep. 13, 2001	Oct. 25, 2001	P7OVQ 31	40	20								
	Oct. 11, 2001	Nov. 22, 2001	P7OVQ 39	20	0								
	Dec. 6, 2001	Dec. 6, 2001	P7OVQ 41	20	0								
		Dec. 20, 2001	P7OVQ 44	20	20								
		Mar. 8, 2002	P7OVQ 62	20	20								
			(+) Control										
			M62	2560	320								
				Jun. 13, 2002	Jun. 13, 2002	Jun. 27, 2002	Jun. 19, 2002	Jun. 21, 2002	Jun. 21, 2002	Jun. 24, 2002	Jun. 24, 2002	Jun. 25, 2002	Jun. 25, 2002
patient 7	Oct. 19, 2001	Oct. 19, 2001	P7OVQ 28	0	0	0	(re-do)	0	0	0	0	20	0
	Oct. 26, 2001	Nov. 16, 2001	P7OVQ 35	640	40	0	(re-do)	0	0	0	0	160	160
	Nov. 2, 2001	Dec. 14, 2001	P7OVQ 42	80	20	0	(re-do)	0	0	0	0	80	160
	Nov. 30, 2001	Jan. 11, 2002	P7OVQ 48	80	20	0	(re-do)	0	0	0	20	80	80
	Jan. 25, 2002	Jan. 25, 2002	P7OVQ 53	80	20	0	(re-do)	0	0	0	20	80	40
		Feb. 8, 2002	P7OVQ 56	80	20	0	(re-do)	0	0	0	20	80	80
		Apr. 19, 2002											
			(+) Control										
			M62	2560	160								
			Positve control				2560						
			S193(1 mg/ml)					6400					
			Positve control						2560				
			Positve control							640			
			Positve control								2560		
			Positve control									2560	2560
			Positve control			2560							
				Jun. 18, 2002	Jun. 18, 2002								
Patient 8	Oct. 23, 2001	Oct. 23, 2001	P7OVQ 29	0	0								
	Oct. 30, 2001	Nov. 20, 2001	P7OVQ 38	0	0								

-continued

Experiment 2: Median ELISA titers and FACS results against MCF-7 and LSC cells after vaccination groups of 5 C57BL/6 Mice with heptavalent-KLH conjugate vaccine

			ELISA			FACS with MCF-7							FACS with LSC	
	Nov. 6, 2001	Dec. 18, 2001	P7OVQ 43	0	0									
	Dec. 4, 2001	Jan. 15, 2002	P7OVQ 51	0	0									
	Jan. 29, 2002	Jan. 29, 2002	P7OVQ 54	40	0									
		OFF TRIAL												
			(+) Control											
			M62	2560	640									
				Jun. 18, 2002	Jun. 18, 2002									
Patient 9	Nov. 6, 2001	Nov. 6, 2001	P7OVQ 34	0	0									
	Nov. 13, 2001	Dec. 4, 2001	P7OVQ 40	1280	80									
	Nov. 20, 2001	Jan. 1, 2002	P7OVQ 45	320	80									
	Dec. 18, 2001	Jan. 29, 2002	P7OVQ 55	320	80									
	Feb. 12, 2002	Feb. 12, 2002	P7OVQ 58	160	40									
		Feb. 26, 2002	P7OVQ 60	160	80									
		May 9, 2002	P7OVQ 64	40	80									
			(+) Control											
			M62	2560	320									
				Jun. 13, 2002	Jun. 13, 2002	Jun. 27, 2002	Jun. 19, 2002	Jun. 21, 2002	Jun. 21, 2002	Jun. 24, 2002	Jun. 24, 2002		Jun. 25, 2002	Jun. 25, 2002
Patient 10	Nov. 20, 2001	Nov. 20, 2001	P7OVQ 37	0	0	0	(re-do)	0	0	0	0		0	0
	Nov. 27, 2001	Dec. 18, 2001	P7OVQ 46	320	80	320	(re-do)	0	20	0	20		160	80
	Dec. 4, 2001	Jan. 15, 2002	P7OVQ 50	320	320	320	(re-do)	0	20	0	20		80	640
	Jan. 1, 2002	Feb. 12, 2002	pt no show	—	—	—	—	—	—	—	—		—	—
	Feb. 26, 2002	Feb. 26, 2002	P7OVQ 59	40	160	80	(re-do)	0	20	0	20		40	320
		Mar. 12, 2002	pt no show	—	—	—	—	—	—	—	—		—	—
		May 21, 2002	pt no show	—	—	—	—	—	—	—	—		—	—
			(+) Control											
			M62	2560	160									
			Positve control				2560							
			S193(1 mg/ml)					6400						
			Positve control						2560					
			Positve control							640				
			Positve control								2560			
			Positve control										2560	2560
			Positve control			1280								

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Experiment 2: Median ELISA titers and FACS results against MCF-7 and LSC cells after vaccination groups of 5 C57BL/6 Mice with heptavalent-KLH conjugate vaccine

				ELISA	FACS with MCF-7						FACS with LSC	
				Jun. 12, 2002	Jun. 12, 2002	Jun. 27, 2002	Jun. 19, 2002	Jun. 21, 2002	Jun. 21, 2002	Jun. 24, 2002	Jun. 24, 2002	Jun. 25, 2002	Jun. 25, 2002
Patient 11	Mar. 7, 2001	Mar. 7, 2001	P7OVQ 1	0	0	0	0	0	0	0	20	20	0
	Feb. 5, 2002	Feb. 5, 2001	P7OVQ 57	0	0	0	0	0	0	0	0	20	0
	Feb. 12, 2002	Mar. 5, 2002	P7OVQ 61	0	20	0	0	0	0	0	0	40	80
	Feb. 19, 2002	OFF TRIAL											
	Mar. 19, 2002												
			(+) Control										
			M62	2560	640								
			Positve control				2560						
			S193(1 mg/ml)					6400					
			Positve control						2560				
			Positve control							640			
			Positve control								2560		
			Positve control									2560	2560
			Positve control			2560							

* Patient received one vaccine before protocol hold, restarted ~1 year later

Protocol # 01-019: Serological analysis of Breast cancer patient vaccinated with hexavalent vaccine

	Vaccine	Sample		ELISA GM2 (10 mcg) (March 2002)		ELISA MUC-1-1 5G (3 mcg) (March 2002)		ELISA LeY Ceramide (10 mcg) (April 2002)		ELISA Globo H Ceramide (10 mcg) (May 2002)		ELISA Tf (3 mcg) (May 2002)		ELISA doSM for Tn (3 mcg) (June 2002)		ELISA sTn (3 mcg)	
Patient #	Date	Date	Serology Top	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
Patient 1	Jan. 16, 2002	Jan. 16, 2002	P7BRQ4	20	0	0	0	0	0	0	0	0	20				
	Jan. 23, 2002	Jan. 23, 2002	P7BRQ9	0	0	0	0	0	0	0	0	0	40				
	Jan. 30, 2002	Jan. 30, 2002	P7BRQ11	0	0	0	0	0	0	0	20	0	40				
		Feb. 13, 2002	P7BRQ19	0	0	40	80	0	0	0	20	320	640				
	Feb. 27, 2002	Feb. 27, 2002	P7BRQ28	0	0	20	80	0	0	0	0	80	1280				
		Mar. 13, 2002	P7BRQ37	0	40	40	80	0	0	0	20	160	1280				
		Apr. 10, 2002	P7BRQ44			80	320	0	0	0	20	80	1280				
	May 22, 2002	May 22, 2002	P7BRQ62									40	640				
		Jun. 5, 2002	P7BRQ67														
Controls					*>2560*												
			(*M62*)	*320*													
			(*MCG170*)			*320*	*2560*										
			(*LeYM12*)			*160*	*2560*										
			Mono Ab (*S193*)					*20*	*80/160*								
			(*GB81*)					*1280*									
			Mono Ab (*VK9*)							*0*	*320*						
			(*P7BRQ17*)							*5120*							
			(*slovin lab wk7*)									*320*	*>2560*				
Patient 2	Jan. 16, 2001	Jan. 16, 2002	P7BRQ5	0	0	0	0	0	0	0	20	20					
	Jan. 23, 2002	Jan. 23, 2002	P7BRQ8	0	0	0	0	0	0	0	40	0	40				
	Jan. 30, 2002	Jan. 30, 2002	P7BRQ12	0	0	0	0	0	0	0	2560	160	640				
		Feb. 13, 2002	P7BRQ20	0	0	80	160	0	0	0	640	160	1280				
	Feb. 27, 2002	Feb. 27, 2002	P7BRQ27	0	80	40	20	0	0	0	640	160	1280				
		Mar. 13, 2002	P7BRQ36	0	160	80	20	0	0	0	320	320	640				
		Apr. 10, 2002	P7BRQ46			80	80	0	0	0	80	160	640				
	May 22, 2002	May 22, 2002	P7BRQ61									160	640				
		Jun. 5, 2002	P7BRQ68														
Controls					*>2560*												
			(*P7BRQ17*)	*320*													
			(*hexavalent*)				*>2560*										
			(*P7BRQ16*)			*80*	*320*										
			(*LeYM12*)			*640*											
			Mono Ab(*S193*)					*0*	*160*								
			(*GB81*)					*1280*									
			Mono Ab(*VK9*)							*0*	*320*						
			(*P7BRQ17*)							*5120*							
			(*slovin lab wk7*)									*320*	*>2560*				
Patient 3	Jan. 7, 2002	Jan. 7, 2002	P7BRQ1	0	0	0	0	0	0	0	0	0	0				
	Jan. 14, 2002	Jan. 14, 2002	P7BRQ3	0	0	0	0	0	0	0	0	0	0				
	Jan. 21, 2002	Jan. 21, 2002	P7BRQ7	0	0	0	320	0	20	0	0	0	80				
		Feb. 4, 2002	P7BRQ17	0	20	80	2560	0	40	0	320	320	2560				

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Protocol # 01-019: Serological analysis of Breast cancer patient vaccinated with hexavalent vaccine																	
	Feb. 18, 2002	Feb. 18, 2002	P7BRQ23	0	0	80	640	0	20	0	640	320	1280				
		Mar. 4, 2002	P7BRQ30	0	0	80	320	0	0	0	320	1280	640				
		Apr. 1, 2002	P7BRQ41			80	80	0	0	0	80	320	320				
	May 13, 2002	May 13, 2002	P7BRQ57							0	20	160	320				
		May 27, 2002	P7BRQ63							0	20						
Controls					*>2560*												
			(*P7BRQ17*)	*320*													
			(*hexavalent*)			*160*	*1280*										
			(*P7BRQ16*)			*320*	*2560*										
			(*LeYM12*)					*20*	*160*								
			Mono Ab(*S193*)					*1280*									
			(*GB81*)							*0*	*320/640*						
			Mono Ab(*VK9*)							*0*	*1280/2560*						
			(*P7BRQ17*)									*320*	*2560*				
			(*slovin lab wk7*)														

Patient Name	Vaccine Date	Sample Date	Serology Top	ELISA GM2 (10 mcg) (March 2002) IgG	IgM	ELISA MUC-1-1 5G (3 mcg) (March 2002) IgG	IgM	ELISA Ley Ceramide (10 mcg) (April 2002) IgG	IgM	ELISA Globo H Ceramide (10 mcg) (May 2002) IgG	IgM	ELISA Tf (10 mcg) (May 2002) IgG	IgM	ELISA dOSM for Tn (3 mcg) (June 2002) IgG	IgM	ELISA sTn (3 mcg) IgG	IgM
Patient 4	Jan. 14, 2002	Jan. 14, 2002	P7BRQ2	0	0	0	0	0	40	0	20	0	40				
	Jan. 21, 2002	Jan. 21, 2002	P7BRQ6	0	0	0	0	0	20	20	80	0	20				
	Jan. 28, 2002	Jan. 28, 2002	P7BRQ10	0	0	0	80	0	40	0	320	20	160				
		Feb. 11, 2002	P7BRQ16	0	0	640	320	0	1280	0	640/1280	40	320				
	Feb. 25, 2002	Feb. 25, 2002	P7BRQ26	0	0	160	40	0	320	0	320	40	640				
		Mar. 11, 2002	P7BRQ34	0	0	160	20	0	640	80	320	160	160				
did not come in		Apr. 8, 2002															
		Apr. 15, 2002	P7BRQ47			320	80	0	320	0	160	160	160				
	May 20, 2002	May 20, 2002	P7BRQ58							0	40	320	160				
		Jun. 3, 2002	P7BRQ66							80	40						
Controls					*>2560*												
			(*P7BRQ17*)	*320*													
			(*hexavalent*)			*320*	*>2560*										
			(*P7BRQ16*)			*160*	*>2560*										
			(*LeYM12*)					*20*	*160*								
			Mono Ab(*S193*)					*1280*									
			(*GB81*)							*20*	*320/640*						
			Mono Ab(*VK9*)							*0*	*1280/2560*						
			(*P7BRQ17*)									*320*	*>2560*				
			(*slovin lab wk7*)														
Patient 5	Feb. 6, 2002	Feb. 6, 2002	P7BRQ15	0	0	0	0	0	0	0	0	0	0				
	Feb. 13, 2002	Feb. 13, 2002	P7BRQ22	0	0	0	20	0	0	0	0	0	0				
	Feb. 20, 2002	Feb. 20, 2002	P7BRQ25	0	0	0	0	0	0	0	0	0	0				
		Mar. 6, 2002	P7BRQ33	0	0	40	40	0	0	0	20	20	40				

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Protocol # 01-019: Serological analysis of Breast cancer patient vaccinated with hexavalent vaccine																	
	Mar. 20, 2002	Mar. 20, 2002	P7BRQ38	0	0	20	40	0	0	0	0	0	40				
		Apr. 3, 2002	P7BRQ42			160	40	0	0	0	0	40	40				
		May 1, 2002	P7BRQ51							0	20	20	20				
	Jun. 12, 2002	Jun. 12, 2002	P7BRQ71														
		Jun. 26, 2002															
Controls					*>2560*												
			(*P7BRQ17*)	*320*													
			(*hexavalent*)			*80*	*160*										
			(*P7BRQ16*)			*640*	*320*										
			(*LeYM12*)					*20*	*160*								
			Mono Ab(*S193*)					*1280*									
			(*GB81*)							*0*	*320/640*						
			Mono Ab(*VK9*)							*0*	*1280/2560*						
			(*P7BRQ17*)									*320*	*>2560*				
			(*slovin lab wk7*)									*320*	*1280*				
Patient 6	Feb. 6, 2002	Feb. 6, 2002	P7BRQ14	0	20	0	0	20	0	0	0	0	0				
	Feb. 13, 2002	Feb. 13, 2002	P7BRQ18	0	20	0	0	0	0	0	0	0	0				
	Feb. 20, 2002	Feb 20, 2002	P7BRQ24	0	40	0	80	0	0	0	160	160	80				
		Mar. 6, 2002	P7BRQ31	0	20	320	320	0	0	0	160	160	320				
	Mar. 20, 2002	Mar. 20, 2002	P7BRQ39	0	20	160	320	0	0	0	80	40	160				
		Apr. 3, 2002	P7BRQ43			320	40	0	0	0	40	160	160				
		May 1, 2002	P7BRQ52							0	40	320	320				
	Jun. 12, 2002	Jun. 12, 2002	P7BRQ70														
		Jun. 26, 2002															
Controls					*>2560*												
			(*P7BRQ17*)	*320*													
			(*hexavalent*)			*80*	*640*										
			(*P7BRQ16*)			*640*	*320*										
			(*LeYM12*)					*0*	*80/160*								
			Mono Ab(*S193*)					*1280*									
			(*GB81*)							*0*	*320/640*						
			Mono Ab(*VK9*)							*0*	*1280/2560*						
			(*P7BRQ17*)									*320*	*2560*				
			(*slovin lab wk7*)									*320*	*1280*				

Patient Name	Vaccine Date	Sample Date	Serology Top	ELISA GM2 (10 mcg) (April 2002) IgG	IgM	ELISA MUC-1-1 5G (3 mcg) (April 2002) IgG	IgM	ELISA LeY Ceramide (10 mcg) (April 2002) IgG	IgM	ELISA Globo H Ceramide (10 mcg) (May 2002) IgG	IgM	ELISA Tf (10 mcg) (May 2002) IgG	IgM	ELISA dOSM for Tn (3 mcg) (June 2002) IgG	IgM	ELISA sTn (3 mcg) IgG	IgM
Patient 7	Feb. 27, 2002	Feb. 27, 2002	P7BRQ29	0	0	0	0	0	0	0	0	0	20				
	Mar. 6, 2002	Mar. 6, 2002	P7BRQ32	0	0	0	0	0	0	0	0	0	0				
	Mar. 13, 2002	Mar. 13, 2002	P7BRQ35	0	20	20	320	0	0	0	0	320	160				
		Mar. 27, 2002	P7BRQ40	0	40	320	320	0	0	0	0	640/1280	160				

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Protocol # 01-019: Serological analysis of Breast cancer patient vaccinated with hexavalent vaccine													
	Apr. 10, 2002	Apr. 10, 2002	P7BRQ45	0	80	320	640	0	0	0	0	640	80
		Apr. 24, 2002	P7BRQ49	0	80	160	320	0	0	0	0	1280	80
		May 22, 2002	P7BRQ60							0	0	640	80
	Jul. 3, 2002	Jul. 3, 2002											
Controls					*>2560*								
			(*P7BRQ17*)	*320*									
			(*hexavalent*)			*80*	*640*						
			(*P7BRQ16*)			*640*	*320*	*0*	*1280*				
			(*LeYM12*)					*0*	*160*				
			Mono Ab(*S193*)					*1280*					
			(*GB81*)							*20*	*320*		
			Mono Ab(*VK9*)							*0*	*1280/2560*		
			(*P7BRQ17*)									*640*	*2560*
			(*slovin lab wk7*)									*640*	*1280*
				(June 2002)		(June 2002)		(July 2002)		(July 2002)			
Patient 8	Apr. 22, 2002	Apr. 22, 2002	P7BRQ48										
	Apr. 29, 2002	Apr. 29, 2002	P7BRQ53										
	May 6, 2002	May 6, 2002	P7BRQ54										
		May 20, 2002	P7BRQ59										
	Jun. 3, 2002	Jun. 3, 2002	P7BRQ65										
		Jun. 17, 2002	P7BRQ72										
		Jul. 15, 2002											
	Aug. 26, 2002	Aug. 26, 2002											
		Sep. 9, 2002											
Controls													
			(*P7BRQ17*)										
			(*hexavalent*)										
			(*P7BRQ16*)										
			(*LeYM12*)										
			Mono Ab(*S193*)										
			(*GB81*)										
			Mono Ab(*VK9*)										
			(*P7BRQ17*)										
			(*slovin lab wk7*)										
Patient 9	Apr. 29, 2002	Apr. 29, 2002	P7BRQ50										
	May 6, 2002	May 6, 2002	P7BRQ55										
	May 13, 2002	May 13, 2002	P7BRQ56										
		May 27, 2002	P7BRQ64										
	Jun. 10, 2002	Jun. 10, 2002	P7BRQ69										
		Jun. 24, 2002											
		Jul. 22, 2002											
	Sep. 2, 2002	Sep. 2, 2002											
		Sep. 16, 2002											

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Protocol # 01-019: Serological analysis of Breast cancer patient vaccinated with hexavalent vaccine	
Controls	
	(*P7BRQ17*)
	(*hexavalent*)
	(*P7BRQ16*)
	(*LeYM12*)
	Mono Ab(*S193*)
	(*GB81*)
	Mono Ab(*VK9*)
	(*P7BRQ17*)
	(*slovin lab wk7*)

Notes
Monoclonals:
Dilutions VK9 1:200; S193 1:400
DOSM
Tested positive by ELISA with Monoclonals 5F4 and HB-TN1 .
OSM
Tested positive by ELISA with Monoclonals 5F4, B72.3, and HB-TN1 .
Shaded region denotes Pre sera (week 1)
All samples were tested in duplicate.
*
for patients Lewis and Smith, Lewis Y plates were coated at 0.3, future plates will be coated at 0.2.
Italics controls previously tested as positive.

Hexavalent Pilot study, protocol number #00-64, ELISA tested against TF(c)-HSA

Ag	Vaccination Patient	Ab	1 wkl	2 2	3 3	4 7	12	19	5 21	31	33
TFc	1	Igm	40	40	40	40	>1280	40	40	40	40
HSA		IgG	20	20	>1280	320	80	80	80	160	320
	2	IgM	NMA	80	160	160	80	40	40	20	20
		IgG	10	NMA	10	40	80	NMA	NMA	20	40
	3	Igm		40							
		IgG		NMA							
	4	IgM	40	20	320	80	80	80	NMA		
		IgG	NMA	NMA	640	80	40	160	320		
	5	IgM	NMA	20	160	20					
		IgG	10	NMA	10	20					
	6	IgM		40	640	640	640	160	80	80	160
		IgG		640	>1280	>1280	>1280	>1280	>1280	>1280	>1280
	7	IgM	NMA	40	80	640	160	80	40	80	80
		IgG	10	320	NMA	NMA	40	20	80	20	80
	8	IgM	NMA	NMA	160	160					
		IgG	NMA	NMA	10	80					
	9	IgM	NMA	NMA	NMA	20	NMA	NMA	NMA	NMA	
		IgG	NMA	NMA	40	NMA	640	80	>1280	160	
	10	IgM	NMA	10	160	160					
		IgG	NMA	NMA	40	20					
	11	IgM		20							
		IgG		NMA							
	12	Igm	10	40	160	>1280	80	640	80	20	160
		IgG	NMA	NMA	NMA	NMA	>640	20	40	40	80
	13	IgM		NMA							
		IgG		NMA							
	14	IgM	20	80	40	80	80	40	40	160	160
		IgG	NMA	40	160	>1280	640	80	80	80	160
	15	IgM	NMA	NMA	20	80	40	40			
		IgG	NMA	NMA	10	40	40	40			
	16	IgM	20	80	>1280						
		IgG	10	10	80						
	17	IgM	NMA	10	NMA	40	40	40	40	40	160
		IgG	NMA	NMA		40	40	80	40	20	40
	18	IgM	40	20	80	640	40	80	80	20	80
		IgG	NMA	NMA	NMA	40	160	40	640	40	40

Positive Controls: IgM(19) 1:1280
IgG (7) 1:640
(−) Human AB Serum
As from Oct. 19, 2001 end pt. Titers:
(+) IgM: 640
IgG: 640
(−) IgM: NMA
IgG: NMA

Hexavalent Pilot study, protocol #00-64 ELISA against glycosylated MUC1-1 (5 sites)

Ag	Vaccination Patient	Number	Ab	1 wkl	2 2	3 3	4 7	12	5 19	21	31
MUC		1	Igm	NMA	40	>1280	>1280	>1280	>1280	>1280	>1280
1-1G5			IgG	NMA	NMA	80	160	>1280	640	>1280	>1280
32mer		2	IgM	NMA	NMA		640	10	NMA	10	20
			IgG	NMA	NMA	10	40	40	20.00	40	160
		3	Igm		20	640					
			IgG		NMA	40					
		4	Igm	NMA	NMA	320.00	>1280	>1280	>1280	640	640
			IgG	NMA	NMA	10	320	160	>1280	>1280	>1280
		5	IgM	10	40	>1280		>1280			
			IgG	NMA	NMA	160		>1280			
		6	IgM		NMA	320	>1280	320	320	160	80
			IgG		NMA	160	>1280	320	160	160	160
		7	IgM	NMA	NMA	40	640	640	80	80	40
			IgG	NMA	NMA	640	>1280	>1280	>1280	>1280	>1280
		8	IgM	NMA	NMA	160					
			IgG	NMA	NMA	10					
		9	IgM	NMA	NMA	40	160	80	20	20	10
			IgG	NMA	NMA	NMA	40	20	10	80	40
		10	Igm	NMA	NMA						
			IgG	NMA	NMA						

-continued

Hexavalent Pilot study, protocol #00-64 ELISA against glycosylated MUC1-1 (5 sites)

	Vaccination			1	2	3	4		5		
Ag	Patient	Number	Ab	wkl	2	3	7	12	19	21	31
		11	IgM	NMA	NMA	10	40	20	160	160	80
			IgG	NMA	NMA	10	320	160	40	40	20
		12	Igm	40	40						
			IgG	NMA	NMA						
		13	Igm	NMA	NMA	20	80	80	320	320	160
			IgG	NMA	NMA	20	20	40	320	320	160
		14	Igm	80	20	160	160	40	40		
			IgG	NMA	NMA	NMA	NMA	40	20		
		15	Igm	NMA	NMA	640	640	640	10	10	10
			IgG	NMA	NMA	640	640	640	160	80	80
		16	Igm	NMA	20						
			IgG	NMA	20						
		17	IgM	NMA	20	320	160	40	640	320	80
			IgG	NMA	NMA	10	80	640	40	640	160

Positive Controls: (7) IgM 1:2560
IgG 1:2560 Oct. 22, 2001
(−) AB Sera
Controls as of Oct. 15, 2001: (+) Igm: >1280
IgG: >1280
(−) IgM: NMA
IgG: NMA

Fourth Series of Experiments

Polyvalent Conjugate Vaccine for Cancer

Preliminary Data of Vaccination of High Risk Breast Cancer (BC) Patients (Pts) with a Heptavalent Antigen—Keyhole Limpet Hemocyanin (KLH) Conjugate Plus the Immunologic Adjuvant QS-21.

We have previously shown that following vaccination with single antigen (Ag)—KLH conjugates plus QS-21, the majority of BC pts generate specific antibody (AB) titers. (Clin Ca Res 6:1693, 2000; PNAS 98(6):3270, 2001; Proc ASCO 16:439a, 1997, 18:439a, 1999, 20:271a, 2001) Single Ag's tested have included MUC-1 (various peptide lengths), sTn clustered (c), GloboH and GM2. In an effort to improve and broaden the immune response, we treated BC pts with seven Ag's: 10 mcg each of GM2, GloboH, Lewisy; and 3 mcg each of TF(c), sTn(c), Tn(c) and glycosylated MUC-1, (32 amino acid (aa) sequence, glycosylated at 5 sites per 20 aa tandem repeat). Each Ag was conjugated to KLH and mixed with 100 mcg of QS-21. Heptavalent vaccines were administered subcutaneously during weeks 1, 2, 3, 7, and 19. We treated ten patients: median age 48 years (range 43-63 yrs); Stage 1V=3, Stage 2 with 4 positive nodes=7. Nine pts have completed immunization. Toxicity was limited to transient grade 2 local skin reactions and grade 1-2 flu-like symptoms. IgM and IgG AB titers were considered positive for each antigen if there was at least an eightfold increase above baseline more than once, during weeks 1-19. Antibody responses are tabulated. (table) MUC1 and TF(c) seem most immunogenic. Flow cytometric analysis (FACS) was obtained pre and post therapy to detect binding of IgM and IgG AB against MCF-7 tumor cells. A positive FACS was defined as at least a threefold increase above baseline and was observed in 6/9 patients for IgM and 0/9 for IgG. Further analyses are ongoing. Our next cohort will evaluate the same antigens conjugated to KLH but with GP-100 as the immunologic adjuvant.

Number of pts with positive AB response/Number of pts evaluable

Ag	GM2	Ley	MUC1	TF(c)	sTn(c)	Tn(c)	GloboH
IgM	2/9	1/9	8/9	8/9	4/9	7/9	6/9
IgG	0/9	1/9	8/9	8/9	1/9	0/9	0/9

Objectives

- Determine immune response against seven antigens and cell lines expressing these antigens
- Evaluate toxicity

Background

- Preclinical data demonstrates that conjugation of an antigen with keyhole limpet hemocyanin (KLH) and addition of the immune adjuvant QS-21 augments immunogenicity (Cancer Immunol Immunother 41:185, 1985; Cancer Res 56:3315, 1996)
- Following vaccination with single antigen—KLH conjugates plus QS-21, most breast cancer patients generated IgM and IgG antibodies against the immunizing antigens (Clin Ca Res 6:1693, 2000; PNAS 98(6):3270, 2001; Proc ASCO 16:439a, 1997, 18:439a. 1999, 20:271a, 2001)
- These single antigens have included MUC-1 (various peptide lengths), sTn clustered (c), GloboH and GM2
- To broaden the immune response, seven antigens were individually conjugated to KLH and mixed with QS-21 to construct this heptavalent vaccine

Vaccine Components•Antigens

- Protein: glycosylated MUC-1 (32aa peptide)
- Gangliosides: GM2, GloboH
- Carbohydrates: Lewisy, sTn(c), Tn(c), TF(c)
- Immunogenic Protein Carrier
- KLH (by the following methods of conjugation):
  - MBS (m-maleimidobenzoyl-N-hydroxysuccinimide ester) linker for TF(c), sTn(c), Tn(c), and MUC1

MMCCH (4-[4-N-maleimidomethyl]cyclohexane-1-carboxyl hydrazide) linker for GloboH and Le^Y

Direct reductive amination for GM2

Immunologic Adjuvant

QS-21 (purified saponin fraction of tree bark)

Vaccine Components

	Doses
Antigens*	
GM2	10 mcg
MUC-1	3 mcg
Lewisy	10 mcg
GloboH	10 mcg
TF(c)	3 mcg
Tn(c)	3 mcg
sTn(c)	3 mcg
Adjuvant	
QS-21	100 mcg

*(each conjugated to KLH)

Treatment and Evaluation Plan

	WEEK #									
	1	2	3	5	7	9	13	19	21	(q 3 months)
VACCINE	1	2	3		4			5		
Blood Samples for Immune Response	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Stage

II (with ≥4+ nodes):	7
IV (NED):	2
IV (stable on hormone tx):	1
	n = 10

Common Toxicities•Grade 1-2 injection site skin reactions

Grade 1-2 flu-like symptoms

No significant laboratory abnormalities

No definite autoimmune reactions

Response Criteria: ELISA

Serologic Response by ELISA (Enzyme-Linked Immunosorbent Assay)

IgM and IgG antibody titers were considered positive for each antigen if there was a≥eightfold increase above baseline more than once, during weeks 1-19

Immunologic Response

Patient was considered a responder if there was a serologic response to at least 3 of the 7 antigens

Response Criteria: FACS and CDC

Response by FACS (flourescence activated cell sorter) was considered positive if there was the following increase above baseline:

≥3-fold increase in percent gated positivity, AND

≥1.5-fold increase on MFI (mean flourescence intensity)

Response by CDC (complement-dependent cytotoxicity) was considered positive if there was a 20% increase above baseline

Immune Response Data ELISA

	GM2		MUC-1		Lewisy		GLOBOH		TF		Tn		sTn	
	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
1	−	−	+	+	−	−	−	−	+	+	−	−	−	−
2	+	−	+	+	−	−	+	−	+	+	+	−	+	−
3	+	−	+	+	−	−	+	−	+	+	+	−	+	−
4	−	−	+	+	+	−	+	+	+	+	+	−	−	−
5	−	−	−	+	−	−	−	−	−	−	−	−	−	−
6	−	−	+	+	−	−	+	−	+	+	+	−	+	−
7	+	−	+	+	−	−	−	−	+	+	+	−	−	−
8	−	−	+	+	−	−	+	−	+	+	−	−	+	−
9	−	−	+	+	−	−	+	−	+	+	−	−	−	+
10	−	−	+	+	−	−	+	−	+	+	−	−	+	−
SUM	3	0	9	10	1	0	7	1	9	9	5	0	5	1

Eligibility Criteria

Breast cancer patients with any one of the following features:

Stage 1V (stable on hormone therapy [tx])

Stage 1V (no evidence of disease [NED])

Stage III

Stage II (≥4 positive nodes)•Ipsilateral breast or axillary recurrence

Rising CA15-3 or CEA levels and NED

Patient Characteristics

Total number of patients treated: 10

Total number of vaccinations completed: 50

*(One patient was delayed for unrelated issues)

Median age: 48 years (range 43-63 years)

Immune Response Data FACS and CDC

	FACS (IgM)			CDC	
Patient	MCF-7	LSC	Du-175	MCF-7	LSC
1	+	+	+	−	−
2	−	+	−	+	+
3	+	−	−	−	−
4	+	+	+	+	+
5	−	−	−	−	−
6	+	+	−	+	−
7	+	+	−	−	+
8	−	+	−	+	−

-continued

	FACS (IgM)			CDC	
Patient	MCF-7	LSC	Du-175	MCF-7	LSC
9	+	+	+	−	−
10	−	−	−	+	−
SUM	6	7	3	5	3

Conclusion

- Vaccination with a heptavalent antigen-KLH conjugate plus QS-21 is well tolerated in breast cancer patients
- IgM and IgG antibody responses (to at least 3 of 7 antigens) were observed in 8 patients and 2 patients respectively
- MUC1 and TF(c) to be appear the most immunogenic of the seven antigens in this vaccine
- IgM antibody binding to tumor cells (MCF-7, LSC, Du-145) by FACS analysis was observed in 6 patients, 7 patients, and 3 patients respectively
- There was no consistent evidence of IgG antibody binding to tumor cells by FACS
- There was evidence of CDC with the MCF-7 and LSC tumor cell lines in 5 patients and 3 patients respectively
- Our next cohort will evaluate the same antigens conjugated to KLH but with GP-100 as the immunologic adjuvant

What is claimed is:

1. A method for inducing antibody production in a subject comprising administering an immunogenic composition comprising an adjuvant and antigens comprising MUC1-Tn conjugate, GloboH, TF(c) and at least one antigen selected from the group consisting of GM2, sTn(c) and sialyl Le^a, wherein said conjugate and each of said antigens are individually conjugated to a carrier.

2. The method of claim **1**, wherein said composition comprises MUC1-Tn conjugate, GloboH, TF(c) and GM2.

3. The method of claim **1**, wherein said composition comprises MUC1-Tn conjugate, GloboH, TF(c) and sTn(c).

4. The method of claim **1**, wherein said composition comprises MUC1-Tn conjugate, GloboH, TF(c) and sialyl Le^a.

5. The method of claim **1**, wherein said composition comprises MUC1-Tn conjugate, GloboH, TF(c), sTn(c) and sialyl Le^a.

6. The method of any one of claims **1-5**, wherein said subject has prostate cancer.

7. The method of any one of claims **1-5**, wherein said subject has breast cancer.

8. The method of any one of claims **1-5**, wherein said subject has ovarian cancer.

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